EASL LIVER CANCER SUMMIT 2023

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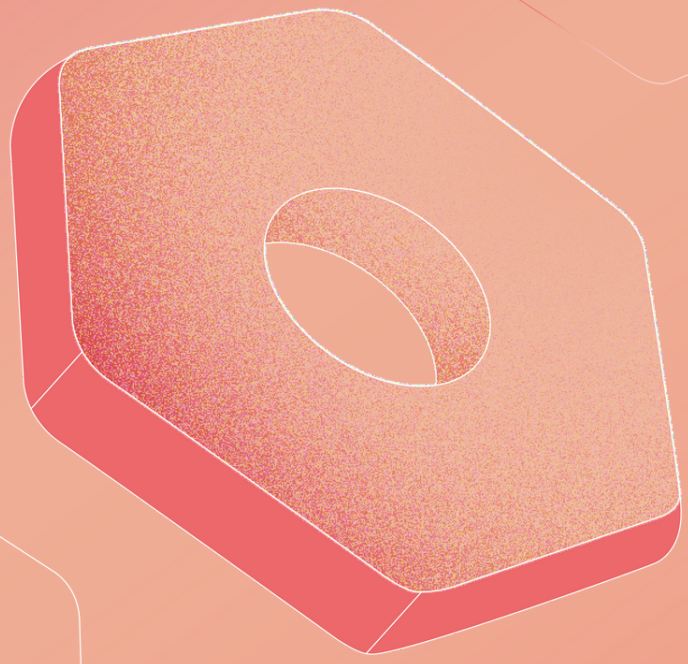

Abstract book

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Content

ORAL ABSTRACT PRESENTATIONS

POSTER ABSTRACT PRESENTATIONS - Basic Science

ORAL ABSTRACT PRESENTATIONS

OS-01-YI
Multiplatform single cell spatial dissection of the invasive front of hepatocellular carcinoma (HCC) reveals molecular insights into tumor progression

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Background and Aims: Cells at the invasive edge of a tumour evade immune surveillance and drive tumour progression, but characterizing these cells in HCC has remained elusive. We performed multi-region profiling of primary HCC and used single cell spatial technologies to identify how heterogenous tumour evolution from the core to the invasive edge drives disease progression.

Method: We obtained 30 tissue samples from HCC resection specimens of 7 patients, under appropriate IRB approval. With a tissue microarray of these samples, we analysed 48,458 cells from three distinct regions: tumour core (15,668 cells), invasive edge (23,234 cells), and uninvolved liver (9,556 cells). We used CODEX, a 42-plex immunofluorescent imaging approach, to identify cell populations and interactions. Spatial transcriptomics (Nanostring GeoMx) determined the expression of 1,812 genes in the tumour compartment (CK+) and microenvironment (TME) (CK-/CD45+). Fluorescent RNA *in situ* hybridization (mRNA FISH) validated gene expression at the single-cell level (**Fig. 1A**).

Results: We identified 20 unique cell types in our samples with CODEX. The invasive edge had a higher proportion of endothelial cells ($p = 0.003$), CD4+ T cells ($p = 0.01$), exhausted CD8+ T cells ($p = 0.04$), and fibroblasts ($p = 0.04$) than the uninvolved liver. Compared to the tumour core, the edge had a higher proportion of CK19+ cancer stem-like cells ($p = 0.03$) and more interactions between these CK19+ cells and exhausted CD8+ T cells ($p = 0.01$), CD4+ T cells ($p = 0.02$), and fibroblasts ($p = 0.04$) (**Fig. 1B**). CD206+/PDL1+ M2-like macrophages and CD8+ T cells also interacted more frequently in the edge ($p = 0.02$).

Comparing the invasive edge vs core, spatial transcriptomics identified 141 differentially expressed genes in the tumour compartment, with upregulation of genes promoting pro-tumoral inflammation (*SERPING1*, *IL6ST, CD81*, *NCOR1*, *PSEN1*). The TME showed differential expression of 38 genes and upregulation of immune modulating genes (*CD164*, *ST6GAL1*, *ITCH*) (**Fig. 1C**). Enrichment analysis revealed TGF-beta to be the top upregulated pathway in the invasive edge (NES 1.46, $p = 0.01$).

Through mRNA FISH, we validated that *TGFB1* mRNA transcripts were indeed more abundant in the invasive edge than the core (47% of edge cells vs. 36% core cells) ($p < 2810^{-16}$) (**Fig. 1D**).

Conclusion: By integrating multiplatform single cell spatial data from multi-region sampling of HCC, we demonstrate remodelling of the immune microenvironment in the invasive edge of the tumour. Compared to the core, the edge is enriched in aggressive CK19+ cancer stem-like cells and exhausted CD8+ T cells that interact with each other and with M2-like macrophages. We validate TGF-beta pathway activation as the master regulator of cancer stemness in the edge. Our spatial analysis provides

mechanistic insights into tumour progression at the invasive edge and identifies molecular targets for therapy.

Figure:

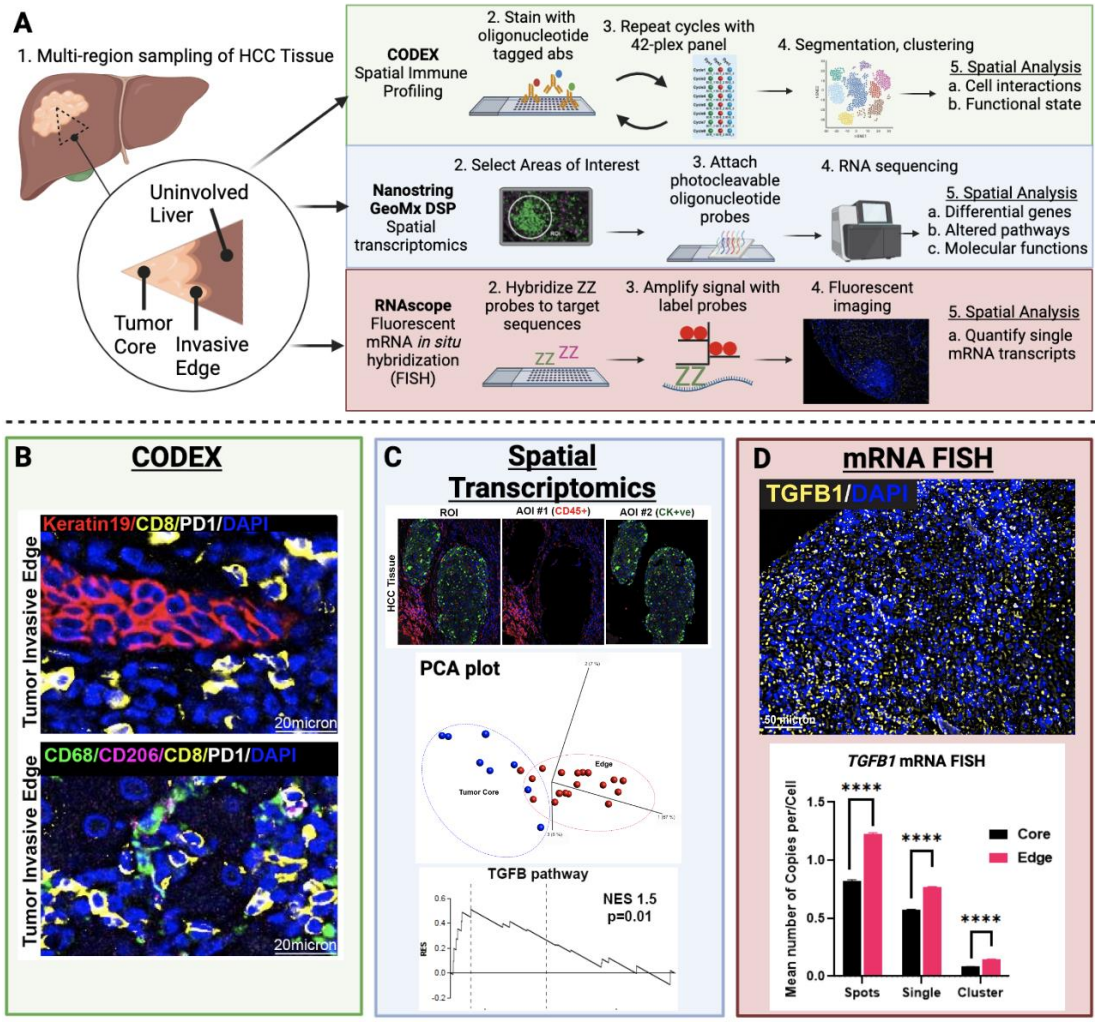

Figure 1. A) Study design: Multi-region sampling of HCC tissue followed by single cell spatial analysis. We use three powerful technologies: CODEX, a 42-plex immunofluorescent imaging approach; Nanostring GeoMx, a spatial transcriptomic platform; and RNAscope, an mRNA FISH assay. B) CODEX reveals enrichment of CK19+ cancer stem-like cells at the invasive edge and their interaction with exhausted CD8+ T cells. C) Spatial transcriptomics determines gene expression in the CK+ tumor compartment and CK-/CD45+ TME. PCA plot indicates separate clusters between tumor core and invasive edge. The TGFβ pathway is the most upregulated pathway along the edge. D) mRNA FISH validates TGFβ upregulation, showing that TGFB1 transcripts are more abundant at the invasive edge than in the tumor core.

OS-02
Radiomics-based prediction of future portal vein tumor infiltration in patients with HCC – a proof-of-concept study

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Background and Aims: Portal vein infiltration (PVI) is a typical complication of HCC. Once diagnosed, it leads to classification as BCLC C with an enormous impact on patient management as systemic therapies are henceforth recommended. Our aim was to investigate whether radiomics analysis using imaging at initial diagnosis can predict occurrence of PVI in the course of disease.

Method: Between 2008 and 2018, we retrospectively identified 44 patients with HCC and an in-house, multiphase CT-scan at initial diagnosis who presented without CT-detectable PVI at diagnosis but developed it in the course of disease. Using propensity score matching accounting for size and number of lesions, growth type, arterial enhancement pattern, Child-Pugh stage, AFP levels, and subsequent therapy, we matched 44 patients with HCC who did not develop PVI to those developing PVI in the course of disease (follow-up ended Dec. 2021). After manual segmentation of the tumor at initial diagnosis, we employed texture analysis and LASSO regression to find radiomics features suitable for PVI detection in this matched set.

Results: After dropping redundant features with high correlation, a total of 47 radiomics features were included in the LASSO regression analysis. Using an 80:20 split between training and holdout validation dataset, 17 radiomics features remained in the fitted model. Applying the model to the holdout validation dataset, sensitivity to detect occurrence of PVI was 0.78 and specificity was 0.78.

Conclusion: Radiomics feature extraction has the ability to detect aggressive HCC morphology likely to result in future PVI. An additional radiomics evaluation at initial diagnosis might be a useful tool to identify patients with HCC at risk for PVI during follow-up benefiting from a closer surveillance.

Figure:

Table 1. Baseline characteristics of the patient groups with and without future PVI [IQR interquartile range, AFP = alpha-fetoprotein, NASH = nonalcoholic steatohepatitis].

Parameter	PVI-negative group (n=44)	PVI-positive group (n=44)	p-value
Age, years [IQR]	65 [59-72]	71 [63-74]	0.05
Number of lesions, n [IQR]	3 [1-6]	4 [2-9]	0.59
Size of lesions, mm, median [IQR]	39 [28-56]	44 [32-68]	0.62
Growth type			
Nodular, n	36	34	
diffuse, n	8	10	0.71
Non-rim arterial enhancement pattern			
hypervascular, n	23	25	
hypovascular, n	4	4	
mixed, n	27	15	0.90
Child-Pugh stage			
A, n	22	26	
B, n	22	17	
C, n	0	1	0.37
AFP levels, ng/ml, mean [IQR]	11946 [16-22316]	15193 [38-43866]	0.45
Etiology			
C2, n	18	21	
chronic hepatitis B, n	8	6	
chronic hepatitis C, n	12	10	
NASH, n	4	3	
Unknown, n	2	4	0.83
Initial treatment*			
curative, n	10	8	
intra-arterial, n	33	35	
systemic, n	1	1	0.87

*curative includes surgery and ablation, intra-arterial includes trans-arterial chemo-embolization and selective internal radiation therapy.

OS-11-02-YI
GALAD score outperforms aMAP and ALBI scores in the 5- and 10-year prediction ofhepatocellular carcinoma (HCC) development in patients with compensated advanced chronicliver disease (cACLD): a 12-year prospective study

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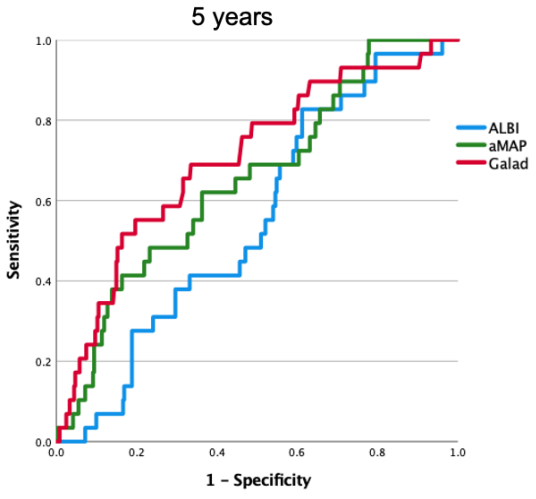
Background and Aims: Several scores for hepatocellular carcinoma (HCC) prediction have been derived from the analysis of prospective or retrospective patients' cohorts, among these aMAP and ALBI scores. In this study, we have tested the performance of GALAD score alone for predicting 5-year and 10-year HCC risk score from the analysis of a cohort of patients with cACLD of any etiology prospectively enrolled since 2011 vs. aMAP and ALBI scores.

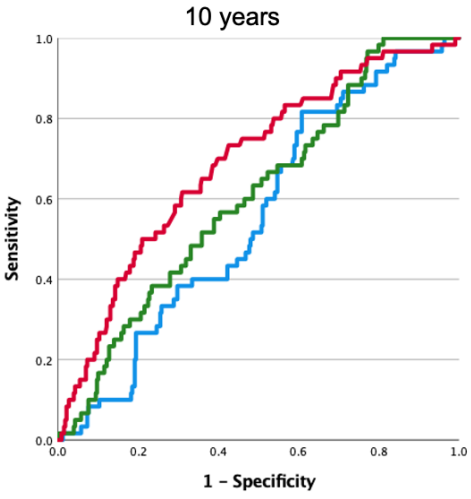
Method: A cohort of 545 patients with cirrhosis was recruited and their GALAD score was calculated. The patients were followed up for a median of 3.60 years at 5 years, and 5.58 years at 10 years. A Cox proportional hazard regression model was used to analyse the predictive ability of GALAD, aMAP and ALBI scores towards the development of HCC at 5 and 10 years.

Results: The results of the study showed that GALAD were all significant predictors of HCC at 5 (HR = 1.373, p<0.001), aMAP (HR = 1.097, p<0.001), and ALBI (HR = 0.162, p = 0.074) and at 10 years (HR = 1.315, p<0.001), aMAP (HR = 1.072, p<0.001), and ALBI (HR = 0.389, p = 0.042) respectively. Their direct comparison at the same time points showed that GALAD outperformed the other two for the prediction both at 5 and at 10 years (Figure).

Conclusion: We have shown in this study that GALAD score outperforms two other already validated and commonly used scores (aMAP and ALBI) for HCC prediction both at 5 and at 10 years. One possible explanation for GALAD advantage resides in the specific GALAD composition, i.e. the inclusion of AFP as well as AFP-L3 and DCP, whose alteration, especially on the long-term, can be of relevant gain vs. scores including biochemical parameters, more related with liver function than with carcinogenic risk thus opening new opportunities for the clinical management and the decision making of patients with cACLD.

Figure:





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Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
ALBI	.551	.047	.359	.458	.644
aMAP	.650	.051	.007	.551	.750
Galad	.705	.051	.000	.605	.805

Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
ALBI	.555	.036	.176	.484	.626
aMAP	.606	.037	.009	.534	.678
Galad	.691	.036	.000	.619	.762

OS-04
Claudin-1 is a driver and therapeutic target for Cholangiocarcinoma

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Background and Aims: Cholangiocarcinoma (CCA) shows an alarming rise in incidence and mortality with unsatisfactory treatment options. Claudin-1 (CLDN1), a member of the tight junction family, is a transmembrane protein mediating cell stemness, plasticity and signaling. The functional role of CLDN1 as a therapeutic target for CCA is unknown. We have previously developed highly specific monoclonal antibodies (mAb) targeting exposed non-junctional CLDN1 exhibiting an excellent safety profile in non-human primates (Roehlen, Saviano et al. Science Translational Medicine 2022). Here, we aimed to explore the role of CLDN1 as an oncogenic driver and therapeutic target for CCA.

Method: Comprehensive CLDN1 expression analyses in patient tissues were performed to evaluate CLDN1 as a therapeutic target. Proof-of-concept studies using CLDN1 mAbs were performed in state-of-the-art mouse CDX and PDX models including models for advanced metastatic disease. Single-cell RNA sequencing and proteomics were applied to investigate tumor cell fate and signaling in vivo and ex vivo models.

Results: Comprehensive analyses of CLDN1 protein and RNA expression in CCA patient tissues revealed a marked and significant upregulation of CLDN1 in CCA. Single-cell RNA sequencing of the CCA microenvironment revealed strong expression in tumor cells showing EMT, cell cycle and interferon response signature, uncovering CLDN1 as a therapeutic target. Targeting exposed CLDN1 by highly specific mAbs resulted in a significant and robust antitumoral effect in vivo across CDX and PDX models for intra- and extrahepatic CCA including advanced metastatic disease. Functional studies in cell-based models of CCA showed that CLDN1 mAbs markedly and significantly suppressed migration and invasion of tumor cells. Mechanistically, treatment with CLDN1 mAb suppressed Notch1, Src, and Hippo-YAP signaling - key signal transduction pathways implicated in CCA development and progression.

Conclusion: Collectively, these results support an important functional role for CLDN1 in CCA pathogenesis and provide robust pre-clinical proof-of-concept for CLDN1-specific mAbs to treat CCA, setting the stage for its clinical development.

OS-05
Systematic-comparative genomic and transcriptomic analyses of HCC, ICC and mixed HCC-ICC

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Background and Aims: Liver cancer is the second leading cause of death around the world. It can be divided into three major groups, hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC) and mixed liver cancer (HCC/ICC). There is no clear boundaries between HCC and ICC, with some subtypes sharing similar biological behavior and immune infiltration state. Compared with HCC, the prognosis of ICC is worse. For patients with advanced unresectable liver cancer, the treatment effect is unsatisfactory. It is crucial to identify different molecular subtypes for optimal treatment options.

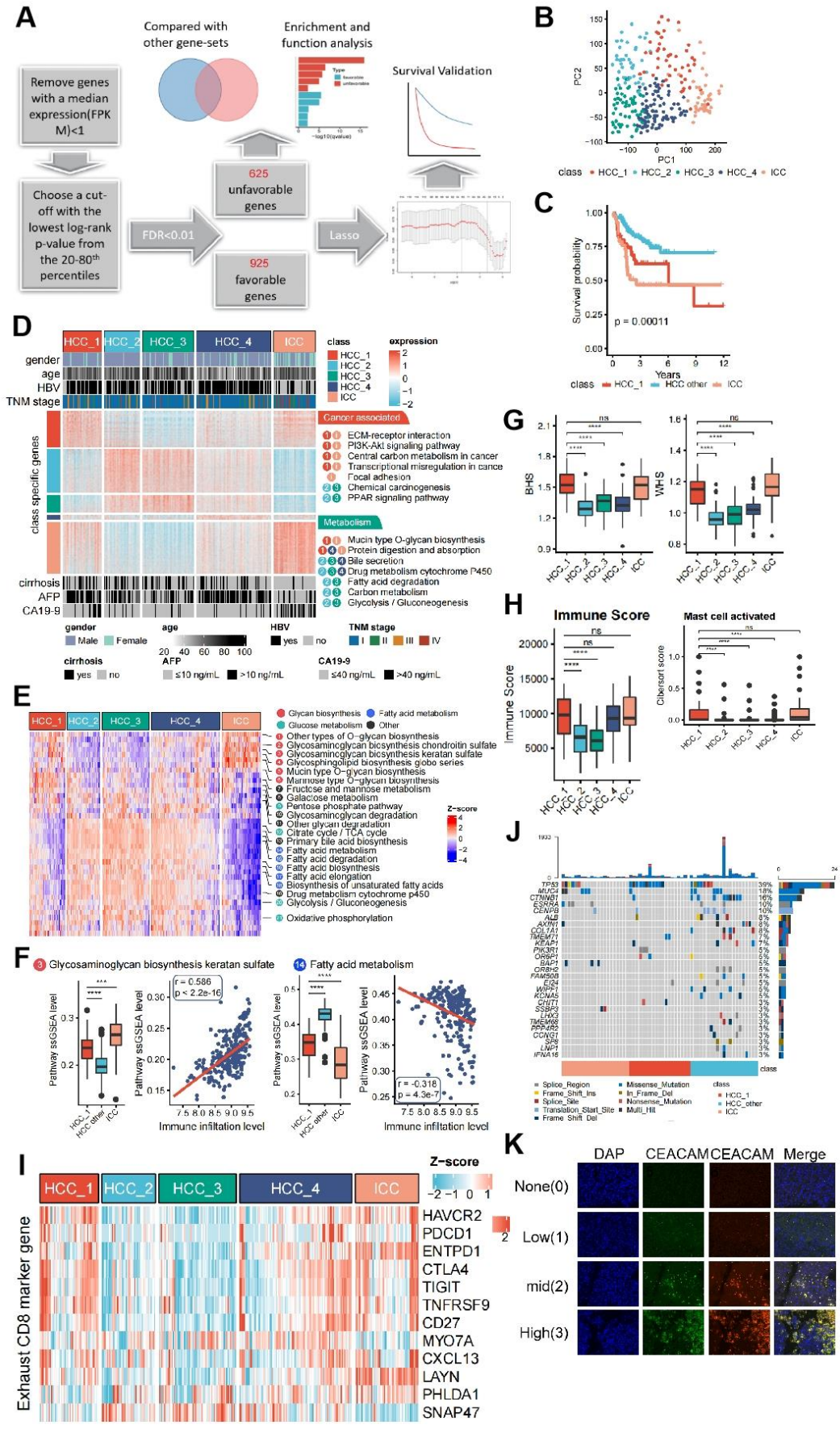
Method: Whole transcriptome sequencing was performed on tumor and adjacent non-tumor tissues across 296 Chinese patients with three types of liver cancer, including HCC, ICC, and combined hepatocellular cholangiocarcinoma (cHCC-CC). Principal component analysis (PCA), weighted gene correlation network analysis (WGCNA), survival analysis, SubMap, single sample Gene Set Enrichment Analysis (ssGSEA), CIBERSORTx, ESTIMATE and gene co-expression network analysis were performed. Whole exome sequencing was performed in a selected subset of samples in each cancer subtype.

Results: The HCC tumors were clustered into 4 subtypes including HCC_1, HCC_2, HCC_3, and HCC_4.

HCC_1 is a novel subtype which is transcriptionally similar to ICC rather than other HCC subtypes, and could hardly summarized by TCGA HCC subtypes. Compared with other HCC subtypes, HCC_1 and ICC showed significant metabolic and immunological commonalities, including high levels of glycan biosynthesis, reduced levels of glucose/lipid metabolism, high levels of hypoxia, highly infiltrated with exhausted CD8+ T cells and activated mast cells. Whole exome sequencing revealed that HCC_1 and ICC had a significant lower tumor mutation burden. Moreover, TP53, PIK3R1, OR6P1, and CHIT1 were identified as the driver genes of HCC_1 subtype. We defined an expression signature to distinguish this distinct HCC subtype, and got tested in TCGA and ICGC data.This study identified a subtype of HCC featured by CEACEM5/6 and FGFR1 with considerably worse prognosis. This distinct subtype might benefit from preventive targeted treatment after surgery.

Conclusion: Our analysis revealed broad similarities between HCC_1 and ICC in metabolism, immune infiltration, hypoxia, gene mutations and overall survival. This will provide a more accurate basis for precision medicine of liver cancer patients in China and East Asia.

Figure:


A
Compared with other gene-sets
Enrichment and function analysis
Survival Validation
Remove genes with a median expression(FPKM)<1
Choose a cut-off with the lowest log-rank p-value from the 20-80th percentiles
FDR<0.01
625 unfavorable genes
925 favorable genes
Lasso
B
PC2
PC1
class HCC_1 HCC_2 HCC_3 HCC_4 ICC
C
Survival probability
p = 0.00011
Years
class HCC_1 HCC other ICC
D
HCC_1 HCC_2 HCC_3 HCC_4 ICC
gender
age
HBV
TNM stage
class specific genes
cirrhosis
AFP
CA19-9
Cancer associated
ECM-receptor interaction
PI3K-Akt signaling pathway
Central carbon metabolism in cancer
Transcriptional misregulation in cance
Focal adhesion
Chemical carcinogenesis
PPAR signaling pathway
Metabolism
Mucin type O-glycan biosynthesis
Protein digestion and absorption
Bile secretion
Drug metabolism cytochrome P450
Fatty acid degradation
Carbon metabolism
Glycolysis / Gluconeogenesis
G
BHS
WHS
H
Immune Score
Mast cell activated
Cibersort score
E
Glycan biosynthesis
Fatty acid metabolism
Glucose metabolism
Other
Z-score
Oxidative phosphorylation
F
3 Glycosaminoglycan biosynthesis keratan sulfate
14 Fatty acid metabolism
Pathway ssGSEA level
r = 0.586
p < 2.2e-16
r = -0.318
p = 4.3e-7
Immune infiltation level
J
TP53
MUC4
CTNNB1
ESR1A
CENPB
ALB
AXIN1
COL1A1
TMEM71
KEAP1
PIK3R1
ORSP1
BAP1
GRB42
FAM50B
EI24
WSR1
KCNA5
CHIT1
SSBP3
LRX3
TMEM98
PPP4R2
CCNG1
SF3
LNP1
IFNA16
class
Splice_Region
Frame_Shift_Ins
Splice_Site
Translation_Start_Site
Frame_Shift_Del
Missense_Mutation
In_Frame_Del
Nonsense_Mutation
Multi_Hit
HCC_1
HCC_other
ICC
I
Z-score
Exhaust CD8 marker gene
HAVCR2
PDCD1
ENTPD1
CTLA4
TIGIT
TNFRSF9
CD27
MYO7A
CXCL13
LAYN
PHLDA1
SNAP47
K
DAP
CEACAM
CEACAM
Merge
None(0)
Low(1)
mid(2)
High(3)

OS-06
Phenotypic characteristics of primary liver cancer in a large French cohort of patients with viral chronic liver disease followed-up before and after viral eradication: an ANRS study

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Background and Aims: In patients with viral chronic liver disease (VCLD), the risk of primary liver cancer (PLC) diminishes significantly after viral eradication but it does not become null. Our study aims to describe the phenotypic characteristics of PLC occurring in a large cohort of patients with VCLD, followed-up before and after viral eradication.

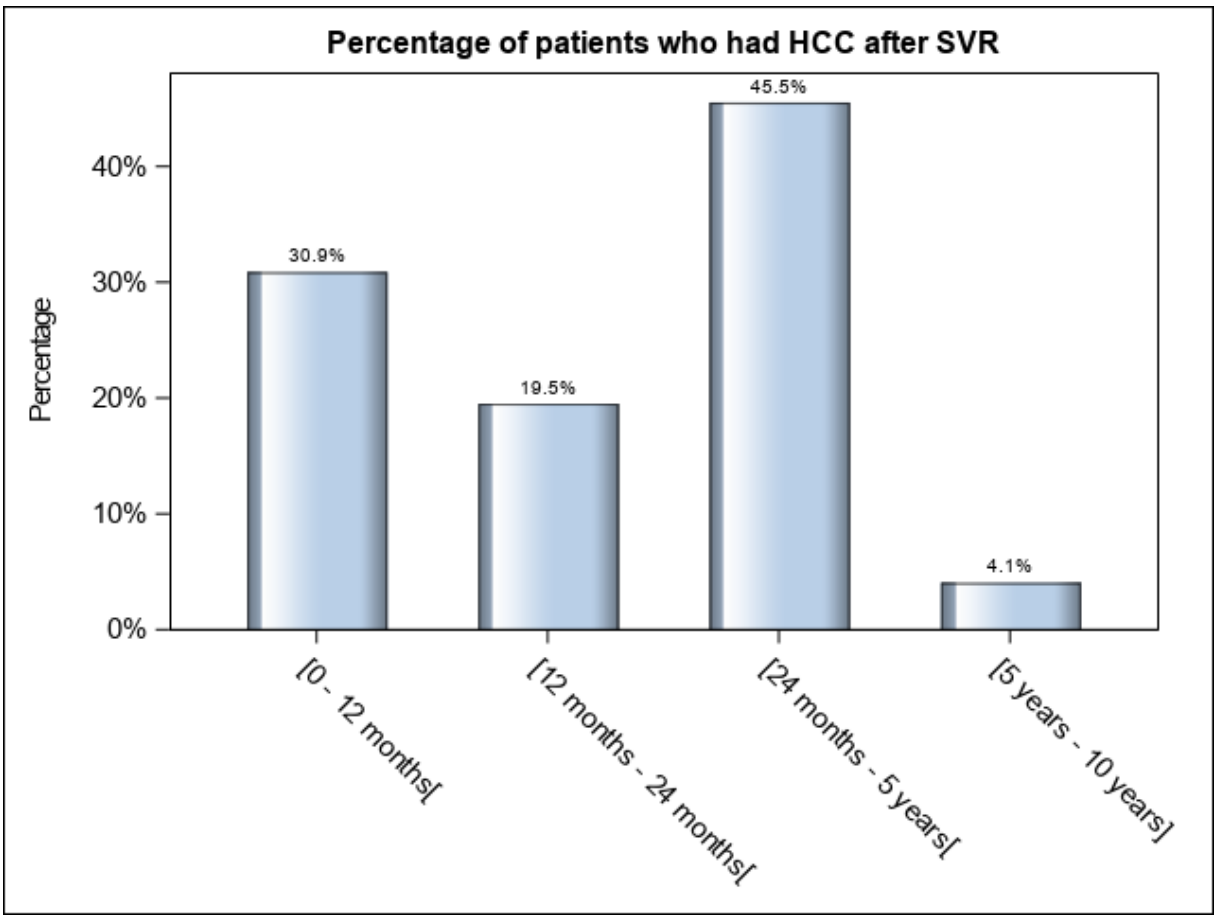
Method: 406 patients with VCLD included in the French cohort LICAVIR (partially prospective), who developed a PLC, were analyzed. Epidemiological, radiological, histological data were recorded and assessed. Patients were followed up at least 61 months [IQR: 40-74]. Statistical analysis was performed using SAS 9.4.

Results: Most patients were males: 312 (77%) with median age of 61.8 years. 368 patients (90%) had hepatitis C virus (HCV) infection, 33 (8%) had hepatitis B virus (HBV) infection and 5 (1%) were coinfected. The main cofactors for chronic liver disease were: alcohol consumption (37%), diabetes (25%), arterial hypertension (35%). 361 patients (89%) had cirrhosis, among whom 82% had Child-Pugh A score and median MELD score of 8.5 [IQR: 7-11]. The median time between cirrhosis and PLC diagnosis was 72 months [IQR: 37-126]. 78% of PLC were diagnosed during regular surveillance. 385 patients (97.8%) developed hepatocellular carcinoma (HCC), 7 patients (1.7%) developed cholangiocarcinoma and 2 (0.5%) patients had hepato-cholangiocarcinoma. At diagnosis, 243 of PLC (62%) were single tumors, with a mean diameter of 28.5 mm, while 67 (17%) were associated with portal vein thrombosis and 22 (6%) were metastatic (27% of lung metastasis). Among HCV-infected patients, 126 developed PLC after achieving sustained virological response (SVR) and were compared to 215 patients who developed it before SVR. Multinodular HCC were more frequent in HCV-infected patients (43% vs 33%, $p < .0001$), while single tumors were more frequent in patients who achieved SVR (66% vs 56%, $p = 0.0075$). The median time between SVR and PLC diagnosis was 23 months [IQR: 9-36]; 96% of PLC occurred within 5 years after SVR. Multiple treatments were performed: among 364 curative treatments, 189 (51%) were percutaneous tumor ablations, 112 (31%) comprised liver resections and

63 (17%) liver transplantations; among 501 palliative treatments, 217 (43%) trans-arterial chemoembolizations and 128 (26%) systemic therapies. During follow-up, 110 patients died.

Conclusion: In our large cohort of patients with VCLD, followed-up before and after viral eradication, PLC developed mostly in cirrhotic liver. The most frequent type of PLC was HCC, which in more than half of patients was a single tumor, detected during a surveillance program. There was no significant phenotypical difference between HCC occurring before or after SVR, except for the multinodular type, which seemed to be more frequent before SVR. In our cohort, most of PLC (96%) occurred within the five first years after SVR.

Figure:



OS-07
A machine learning enabled score based on large varices predicts 5- and 10-yearhepatocellular carcinoma (HCC) development in a 12-year prospective cohort of patients withcompensated advanced chronic liver disease

Sara Ascari[1], Rodolfo Sardone[2], Fabio Castellana[2], Filippo Schepis[1], Valentina Baldaccini[1], Filippo Semellini[1], Alessandra Pivetti[1], Lorenza Di Marco[1], Barbara Lei[1], Nicola De Maria[1], Francesco Dituri[2], Gianluigi Giannelli[1], Erica Villa[1]

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Background and Aims: Most scores for HCC prediction can assess at most 3- or 5-year HCC risk, as the observation period of the derivative cohort is usually short. We aimed to develop a 5- and 10-year HCC risk score from a prospective cohort of patients with compensated advanced chronic liver disease (cACLD) of any aetiology followed up for 12 years.

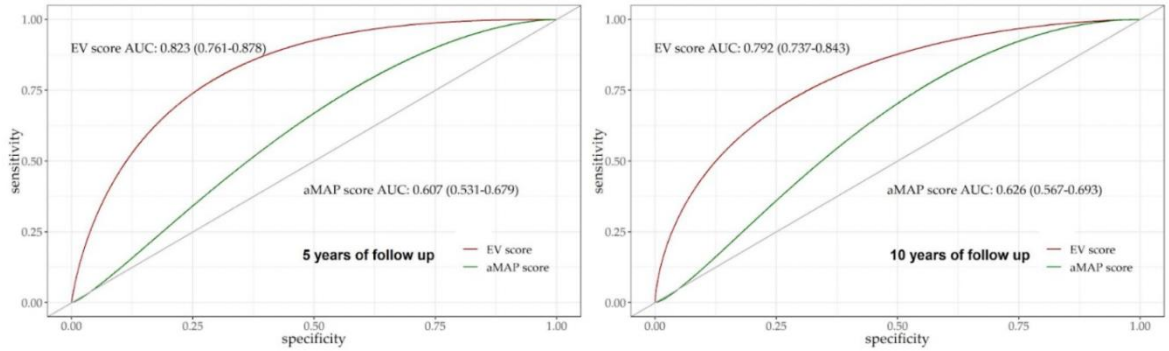
Method: 545 HCC-free patients with cACLD,prospectively enrolled from 2011 to 2022, using a convenience sampling, underwent at enrolment upper g.i. endoscopy, liver ultrasound/elastography,HVPG measurement, lab tests. Cox proportional models were used to assess the association between esophageal varices, adjusted for all the selected covariates, and HCC incidence. Random Survival Forest (RSF), a machine learning (ML) prediction model, was used as a sensitivity analysis to test prediction power of the same covariates, considering all the possible interactions and non-linear relationships with HCC incidence as the outcome.

Results: Median fup time was 5.9 years. We observed 78 incident HCCs (14.3%).In the fully adjusted Cox proportional models after the adjustment for covariates, pts. with large esophageal varices had 4 times the risk of developing HCC (HR:4.02;95% C.I.: 2.42-6.68) than patients with small/without varices. The covariates, viral aetiology (HR 2.61;95% C.I.:1.57-4.35),LSM (for each kPa)(HR:1.01;95 % C.I. 1.01-1.03, male sex (HR:1.94 C.I. 95%: 1.10-3.41), were also meaningfully associated with HCC risk.

As a sensitivity analysis we performed the RSF selection algorithm to rank all the variables of the Cox models, according to their prediction power (using minimal depth metric) for the incidence of HCC. Large esophageal varices had the best prediction power for HCC, followed by LSM,viral aetiology, BMI, albumin, and age at the enrolment. Interestingly, RSF prediction power was in line with the magnitude of association with Cox model, but ML further identified BMI & albumin as related and excluded sex. The score built with the RSF-selected variables (**E**sophageal **V**arices [EV] score) had excellent discrimination and calibration in assessing both 5- (AUROC 0.823) and 10-year (AUROC 0.792) HCC risk irrespective of aetiology, with a significantly better overall performance at both time points than aMAP score, built on the same data (figure).

Conclusion: The machine learning approach, used to build this score, allowed us to identify large varices as the most important predictor for HCC risk (underlining the critical pathogenetic role of longstanding and severe portal hypertension in HCC development). This score also obtained better prediction for 5- and 10-year HCC development than aMAP score (i.e. the best score so far for HCC prediction independently from aetiology) tested in the same dataset. The proposed score is highly reliable. Being based on routine clinical data of the patients with cACLD it can be easily applied worldwide.

Figure:


EV score AUC: 0.823 (0.761-0.878)
aMAP score AUC: 0.607 (0.531-0.679)
5 years of follow up
EV score
aMAP score
sensitivity
specificity
1.00
0.75
0.50
0.25
0.00
EV score AUC: 0.792 (0.737-0.843)
aMAP score AUC: 0.626 (0.567-0.693)
10 years of follow up
EV score
aMAP score
sensitivity
specificity

OS-08
IMbrave050: Phase 3 study of adjuvant atezolizumab + bevacizumab versus active surveillance in patients with hepatocellular carcinoma (HCC) at high risk of disease recurrence following resection or ablation

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Background and Aims: The risk of HCC recurrence after liver resection or ablation with curative intent is 70-80% within 5 years, indicating an unmet need for effective adjuvant therapies. Atezolizumab (atezo) with bevacizumab (bev) is the standard of care for unresectable HCC based on the IMbrave150 study, which demonstrated statistically significant and clinically meaningful improvements in overall survival (OS), progression-free survival, and objective response rate versus sorafenib (Finn NEJM 2020, Cheng J Hepatol 2022). On the basis of the antitumor activity of atezo + bev and its capacity to positively modulate the tumor microenvironment, IMbrave050 was designed to evaluate the efficacy of adjuvant atezo + bev in delaying or preventing recurrence in patients (pts) with high-risk HCC.

Method: IMbrave050 (NCT04102098) enrolled pts with HCC at high risk of recurrence following resection or ablation. High-risk criteria were based on tumor burden (tumor size and number), vascular invasion, and tumor differentiation. Pts were randomized to Arm A (atezo + bev) or Arm B (active surveillance). Stratification factors included geographic region (Asia-Pacific excluding Japan vs rest of world) and a composite factor encompassing the number of high-risk features, curative procedure, and use of optional adjuvant TACE (allowed for one cycle following resection). Pts in Arm A received atezo 1200 mg + bev 15 mg/kg IV q3w for a period of one year or 17 cycles. Pts in Arm B underwent active surveillance for one year and were eligible to crossover to atezo + bev following independent review facility (IRF) confirmation of recurrence. The primary endpoint was IRF-assessed recurrence-free survival (RFS). Secondary efficacy endpoints included OS; investigator-assessed (INV) RFS; RFS and OS according to PD-L1 status; and time to extrahepatic spread and/or macrovascular invasion.

Results: The ITT population included 334 pts each in Arms A and B. Baseline demographics were well balanced between arms. At interim analysis, with a median follow-up of 17.4 mo (cut off date: Oct 21 2022), the primary endpoint was met with an IRF-RFS HR of 0.72 (95% CI, 0.56, 0.93; *P*=0.0120), and results were generally consistent across clinical subgroups. INV-RFS was similar (HR, 0.70; 95% CI,

0.54, 0.91). The safety of atezo + bev was generally manageable and consistent with the well-established safety profile of each therapeutic agent and with the underlying disease.

Conclusion: Atezo + bev is the first adjuvant regimen to demonstrate a statistically significant and clinically meaningful improvement in RFS vs active surveillance in pts at high risk of disease recurrence following resection or ablation. The benefit:risk profile of atezo + bev favors the use of this regimen as an adjuvant therapy and has potential to set a new standard of care in adjuvant HCC.

This abstract was previously presented at the AACR Annual Meeting 2023.

OS-09-YI
Identification of Atezolizumab plus Bevacizumab prognostic index via recursive partitioning analysis in advanced hepatocellular carcinoma: the ABE index

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Background and Aims: This study aims to identify a new prognostic index by applying recursive partitioning analysis (RPA) in hepatocellular carcinoma (HCC) patients treated with atezolizumab plus bevacizumab (AB).

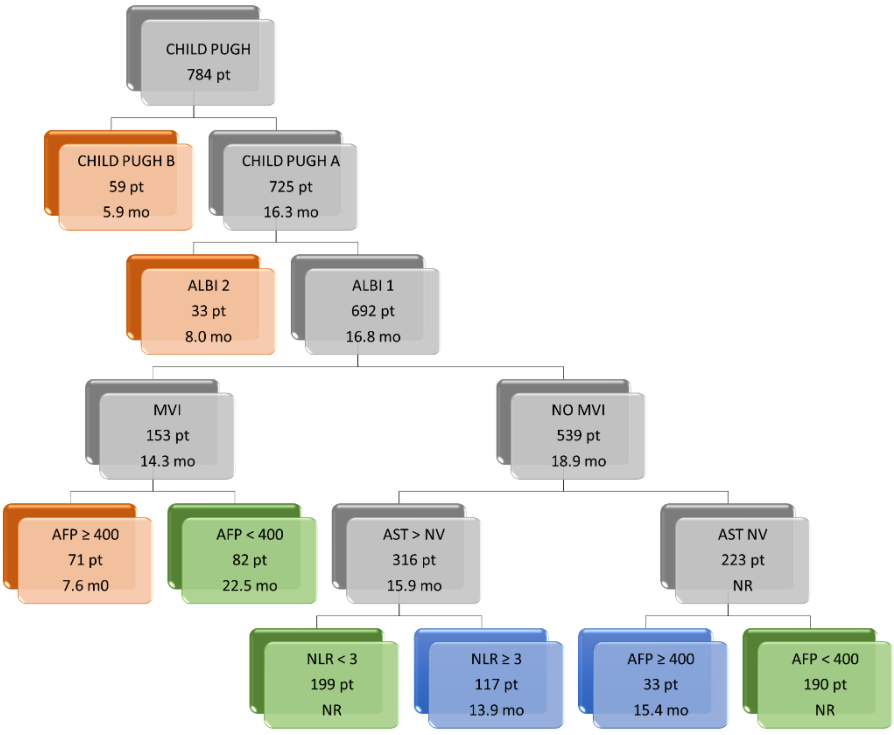
Method: RPA was applied on 784 consecutive HCC patients treated with AB.

Results: RPA allowed the identification of the Atezolizumab Bevacizumab prognostic (ABE) index, comprising three groups of patients: low risk, [(i) Child-Pugh A (CPA) patients without macrovascular invasion (MVI) but with Albumin-Bilirubin (ALBI) 1, aspartate aminotransferase (AST) normal value (NV), and alpha-fetoprotein (AFP) < 400 ng/mL, (ii) CPA patients without MVI but with ALBI 1, AST increased value (IV), and neutrophil-lymphocyte ratio (NLR) < 3, and (iii) CPA patients with MVI, ALBI 1, and AFP < 400 ng/mL]; intermediate risk, [(i) CPA patients without MVI but with ALBI 1, AST NV, and AFP ≥ 400 ng/mL, and (ii) CPA patients without MVI but with ALBI 1, AST IV, and NLR ≥ 3]; high risk [(i) CPA patients with ALBI 2, (ii) CPA patients with ALBI 1, MVI, and AFP ≥ 400 ng/mL, and (iii) CPB patients]. Overall survival was 22.5 months (95% CI 17.0-22.5 months) in patients with low risk (60.1%), 14.2 months (95% CI 12.4-15.7 months) in intermediate risk (19.1%), and 7.0 months (95% CI 6.0-8.7 months) in high risk (20.8%); low risk HR 1, intermediate risk HR 1.76 (95% CI 1.26-2.46), high risk HR 3.99 (95% CI 2.76-5.77); P < 0.01. Progression-free survival was 9.4 months (95% CI 8.4-10.8 months) in patients with low risk, 6.1 months (95% CI 5.5-8.1 months) in intermediate risk, and 5.3 months (95% CI 3.7-5.8 months) in high risk; low risk HR 1 (reference group), intermediate risk HR 1.47 (95% CI 1.14-1.89), high risk HR 1.79 (95% CI 1.37-2.35); P < 0.01. In the three groups, differing profiles of toxicity

have been highlighted, notably in terms of hypertension (low risk 27.4%; intermediate risk 22.7%; high risk 17.2%, P = 0.03), proteinuria (low risk 28.7%; intermediate risk 35.3%; high risk 22.7%, P < 0.05), and hypothyroidism (low risk 6.1%; intermediate risk 2.7%; high risk 1.8%; P = 0.03).

Conclusion: the ABE index is an easy-to-use tool able to stratify HCC patients undergoing first-line therapy with AB.

Figure:



POSTER ABSTRACT PRESENTATIONS

Basic Science

P01-07-YI
Silencing CNNM4 in cholangiocarcinoma inhibits tumoral progression by means of non-canonical ferroptosis

Maria Mercado-Gómez[1], Alvaro Eguilero Giné[1], Miren Bravo[1], Mikel Azkargorta[2 3], Marina Serrano-Macia[1], Naroa Goikoetxea[1], Irene González-Recio[1], Sofia Lachiondo-Ortega[1], Claudia Gil-Pitarch[1], Marta Romero[3 4], Judit Domenech Omella[5], Rocio IR Macias[3 4], Rubén Rodríguez Agudo[1], Leidy Estefanía Zapata-Pavas[1], Patricia Peña-San Felix[1], Paula Olaizola[3 6], Pedro Miguel Rodrigues[3 6 7], Luis Alfonso Martinez-Cruz[1], Angela Lamarca[8 9], Victor Moreno[10], Jesus Maria Banales[3 6 7 11], Teresa Cardoso Delgado[1], Felix Elortza[2 3], Matías A Avila[3 12], Francisco Javier Cubero[3 13 14], Cesar Augusto Martín[15], Miguel Angel Merlos Rodrigo[16], Diego Calvisi[17], Jose Marin[3 4], María Luz Martínez-Chantar[1 3]
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Background and Aims: Cholangiocarcinoma (CCA) is a heterogeneous neoplasm of biliary ducts that represents the second most common primary hepatic cancer, after hepatocellular carcinoma. Due to its aggressiveness, late diagnosis and immunoregulatory capacity of the disease, CCA outcomes are poor, with a median overall survival of less than 12 months. Currently, the only curative treatment is surgical resection, but this only applies to 25% of cases and despite this tumoral recurrence is frequent. For that reason, the study of new therapies is of utmost importance. Recent studies show that the isocitrate dehydrogenase 1 (*IDH1*) inhibitor, used to treat patients with irresectable iCCA harboring *IDH1* mutations, reduces cell proliferation, invasion and metastasis by promoting, ferroptosis, a programed cell death caused by iron-dependent lipid peroxidation.

Results: In this study, we analyze the role of CNNM4 (*Cyclin and CBS Domain Divalent Metal Cation Transport Mediator 4*), a Mg^{2+} effluxer, that is overexpressed in CCA in *in silico*, at transcriptional levels and also in human biopsies. Silencing CNNM4 in CCA human cell lines, EGI-1 and TFK-1, which show high expression of CNNM4, not only increases intracellular Mg^{2+} but also reduces cellular proliferation and sensitizes cells to chemotherapeutic drugs. Key metastasis steps (intravasation, extravasation and invasion in other organs) were also slowed down when CNNM4 is silenced, as seen by 3D spheroid experiments and in *ex ovo* and *in ovo* chicken embryo chorioallantoic membrane assay. Proteomic analysis reveals a metabolic shift into a less glycolytic phenotype in CNNM4-silenced cells, also indicating a role of this transporter in the Warburg effect. Alteration of iron metabolism after CNNM4 modulation in both cell lines is associated with a decrease of NUPR-1 levels, a ferroptosis inhibitor, that

can be a possible mechanism of those effects. In a CCA murine model (myr-AKT/Yap^{S127A}), silencing CNNM4 after tumoral development, via a liver-specific molecule, produces its reversion.

Conclusion: In conclusion, silencing CNNM4 is a potential therapeutic target in CCA whose effect could be mediated by iron-dependent cell death.

P01-08-YI
MiR-494 induces metabolic reprogramming through G6pc targeting and modulates sorafenib response in hepatocellular carcinoma

Ilaria Leoni[1 2], Giuseppe Galvani[1 2], Christian Bergamini[3], Nicola Rizzardi[3], Mattia Melli[3], Camelia Alexandra Coada[4], Elisa Monti[1 2], Catia Giovannini[1 5], Irene Liparulo[3], Manuela Ferracin[5], Matteo Ravaioli[4 6], Matteo Cescon[4 6], Francesco Vasuri[7], Fabio Piscaglia[4 8], Massimo Negrini[9], Claudio Stefanelli[2], Romana Fato[3], Laura Gramantieri[8], Francesca Fornari[1 2]

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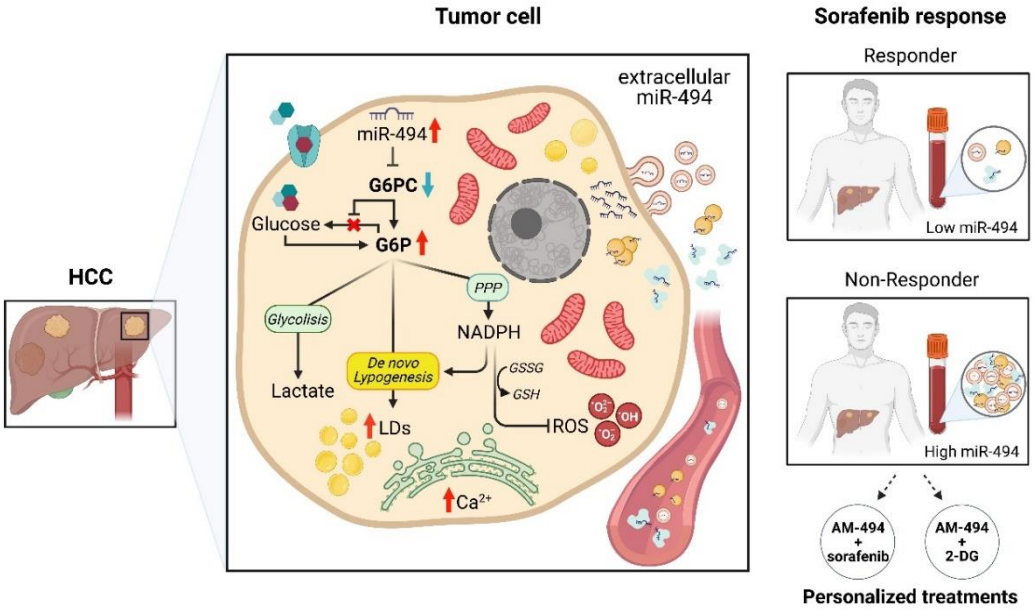
Background and Aims: Metabolic reprogramming is a well-known marker of cancer and it represents an early event in hepatocellular carcinoma (HCC) development. The epidemiology of HCC is increasingly related to metabolic syndrome, which has been rising in developed countries. The recent approval of several molecular targeted agents, including immunotherapy, has revolutionized the management of advanced HCC patients. Nevertheless, the lack of circulating biomarkers still affects patient stratification to tailored treatments, as evidenced by reduced sensitivity to immunotherapy in metabolic-driven HCCs. In this context, there is an urgent need for biomarkers to aid treatment choice and more effective therapeutic combinations avoiding the development of drug-resistant phenotypes. The aims of this study are to investigate the involvement of microRNA-494 (miR-494) in HCC metabolic reprogramming, to identify novel miRNA-based therapeutic combinations and to evaluate miR-494 potential as a circulating biomarker to stratify patients to sorafenib treatment.

Methods: A bioinformatics investigation allowed the identification of miR-494 metabolic targets. Functional and metabolic assays demonstrated glucose-6-phosphatase catalytic subunit (G6pc) targeting by miR-494 and its involvement in metabolic shift, mitochondrial dysfunction, and ROS production in HCC cells. Live-imaging analysis and rescue experiments elucidated the involvement of miR-494/G6pc axis in HCC cell growth under stressful conditions. Circulating miR-494 levels were assayed in serum samples from sorafenib-treated HCC patients and DEN-HCC rats.

Results: We reported miR-494 involvement in HCC cells metabolic shift toward a glycolytic phenotype, which is mainly mediated by G6pc direct targeting. We showed that miR-494/G6pc axis exhibits an active role in metabolic plasticity of cancer cells, regulating glycogen and lipid droplets accumulation to be used as nutrient supply under detrimental conditions. High miR-494 serum levels associated with sorafenib resistance in a small cohort of advanced HCC patients and with metabolic features in surgically-treated patients. AntagomiR-494-based strategies showed a synergistic effect with both metabolic inhibitors and sorafenib in HCC cells.

Conclusions: MiR-494/G6pc axis is critical for the metabolic rewiring of cancer cells and associates with poor prognosis. MiR-494 deserves attention as a candidate biomarker of likelihood of sorafenib response to be tested in future studies. Preliminary data suggest miR-494 as a promising target for combined treatments with sorafenib or metabolic interference molecules for the treatment of HCC patients being ineligible for immunotherapy.

Figure: Schematic representation of miR-494/G6pc axis involvement in metabolic plasticity of HCC cells and therapeutic potential of combined antagomiR-494-based strategies.



P01-09
Developing new therapies for primary liver cancer with precision bio-printed patient-derived organoids

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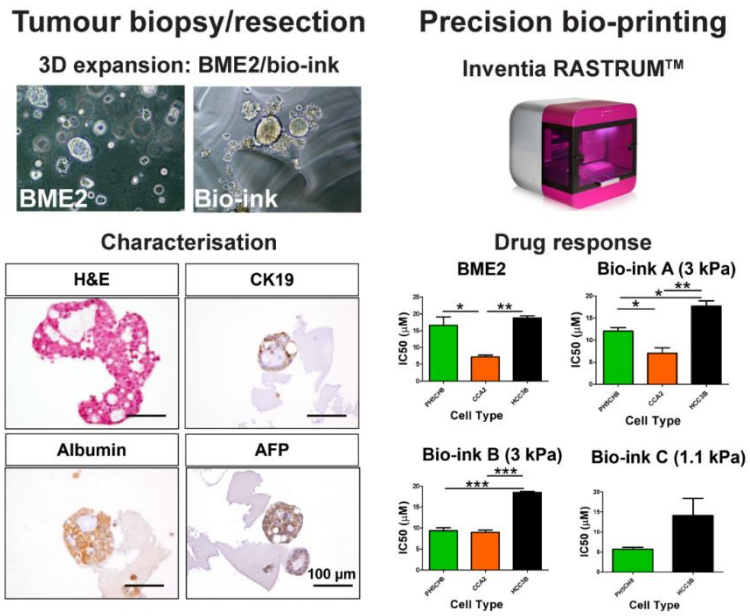
Background and Aims: Liver cancer is the third-leading cause of cancer-related deaths worldwide. Currently available systemic treatments have only modest efficacy and significant associated toxicity. Targeted, more effective therapeutic options are urgently required to ease the burden of disease. Lack of appropriate *in vitro* models is a major obstacle in drug development. Patient-derived organoid (PDO) technology may overcome limitations of conventional *in vitro* models. The reported rate of liver tumour PDO generation is low in non-defined matrices, which do not accurately model the tumour microenvironment. We aimed to develop a liver cancer PDO drug screening system using fully defined bio-inks developed by Inventia Life Science on the RASTRUM™ bio-printer.

Method: 34 primary liver cancer percutaneous biopsy or resection samples have been processed (n = 32 HCC, n = 2 CCA). Tumours were enzymatically digested and plated in Cultrex Basement Membrane Extract (BME2) and/or defined bio-inks (Inventia Life Sciences) to generate PDOs. PDOs were bio-printed using the RASTRUMTM bioprinter (Inventia). Three novel bio-inks with 1.1 or 3 kPa stiffness were used for bio-printing to define a matrix supporting optimal liver cancer PDO growth using high content imaging and biochemical assays. Response to Sorafenib treatment was characterised in two tumour PDO lines and compared to a non-tumour hepatocyte cell line in different precision bio-printed bio-inks and BME2.

Results: 15 PDO lines have been generated. Liver tumour PDOs were successfully bio-printed and utilised in drug response assays. Significant matrix-dependent drug responses in a hepatocyte cell line were observed. We established that a novel, defined 3 kPa bio-ink optimally supports liver tumour PDO growth and can be used in high-throughput 384-well assays.

Conclusion: We have established a PDO biobank from primary liver cancer patients that can be used for drug screening/repurposing experiments. In collaboration with Inventia Life Sciences, we have developed high-throughput precision bio-printed PDO models of primary liver cancer that will support accurate and reproducible high-throughput drug screening to enable novel drug development for primary liver cancer patients.

Figure:


Tumour biopsy/resection
3D expansion: BME2/bio-ink
BME2
Bio-ink
Characterisation
H&E
CK19
Albumin
AFP
100 µm
Precision bio-printing
Inventia RASTRUM™
Drug response
BME2
Bio-ink A (3 kPa)
Bio-ink B (3 kPa)
Bio-ink C (1.1 kPa)
IC50 (µM)
Cell Type

P01-10-YI
The tumor milieu of Intrahepatic Cholangiocarcinoma: dissecting the phenotypical and molecular properties of B lymphocytes in affected patients

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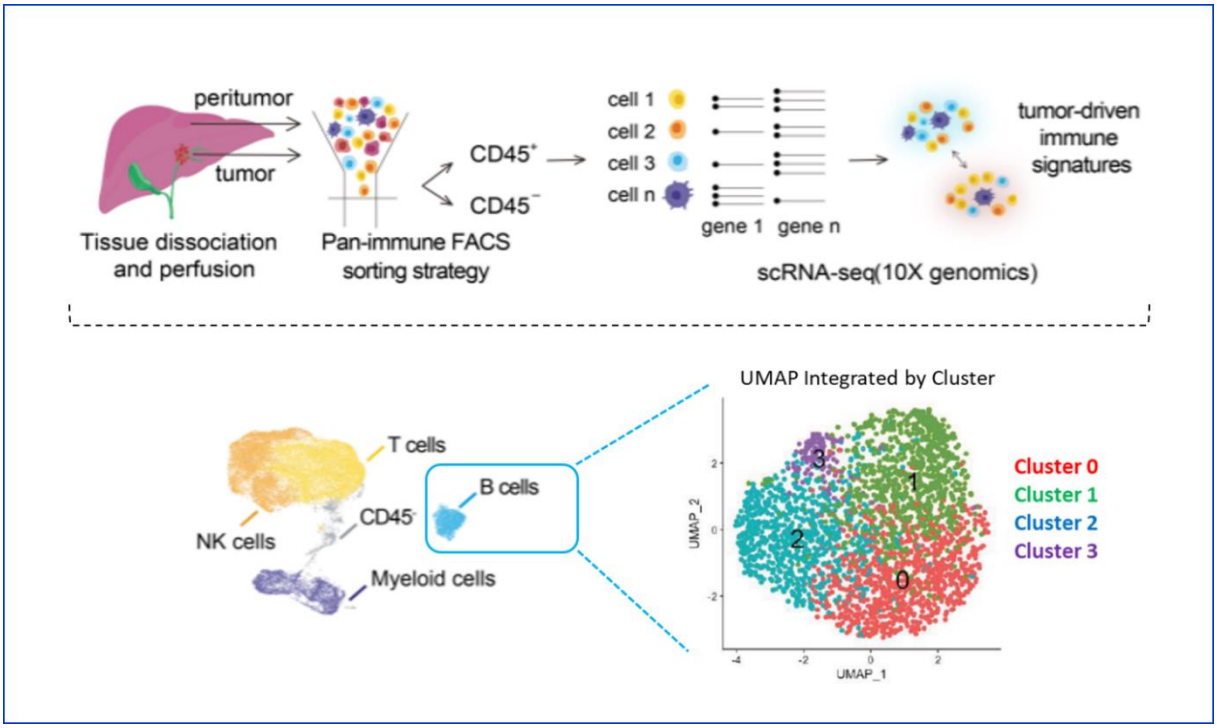
Background and Aims: Intrahepatic cholangiocarcinoma (iCCA) is a heterogeneous biliary tract cancer whose incidence rate increased over the past decades. Due to the aggressive evolution of the disease, there is an urgent need for diagnostic and therapeutic alternatives. The immune infiltrate is a key component of the tumour microenvironment (TME), but remains poorly characterized, limiting the development of successful immunotherapies. Many aspects related to T cells are undergoing extensive studies, while the effect exerted by B lymphocytes in iCCA development and progression is still controversial and unwell characterized. Herein, our aim is dissecting the phenotypic and functional properties of B cells in the TME of iCCA, with the goal of finding new mechanisms important for cancer initiation and/or progression.

Method: We characterized the B-cell compartments of iCCA tissues**,** adjacent tumor-free tissues and circulating counterparts performing high-dimensional single-cell technologies. We further carried out gene expression analysis and cellular assays to define the B cell-specific role in iCCA tumor tissues, investigating whether and how liver TME impact on B cell biology.

Results: Data obtained from single-cell RNA-sequencing analysis of $CD20^{+}$ cells in six iCCA patients identified four main subclusters of B lymphocytes and revealed that genes involved in neutrophil degranulation, cellular response to stress, GPCR binding were up-regulated; on the other hand, genes related to B cell activation/inflammation were downregulated in intratumoral area compared to adjacent non-malignant tissue; suggesting an immunosuppressive role of B cells in iCCA TME. Multicolour flow cytometry analysis of B cells isolated from iCCA patients (n =13) highlighted a higher frequency of naïve B cells respect to the memory phenotype. A reduction in B-cell effector functions was also detected. Immunohistochemical analyses showed that B cells, when infiltrate the tumour, are well organized and create cellular aggregates similar to tertiary lymphoid structures.

Conclusion: Overall, these results suggest that various B cells subtypes can be found in iCCA tissue, probably with an immunosuppressive role. However, a comprehensive characterization of B cell properties, functions, organization and crosstalk with other cells of iCCA milieu will elucidate mechanisms of tumour progression/control, exploitable for the development of novel immunotherapeutic strategies.

Figure:



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P01-13
PRAME is a target of Gas6/Axl signaling in hepatocellular carcinoma

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Background and Aims: Activation of the receptor tyrosine kinase Axl by Gas6 fosters oncogenic effects in hepatocellular carcinoma (HCC), associating with increased mortality of patients. The impact of Gas6/Axl signaling on the induction of individual target genes in HCC and its consequences is an open issue.

Method: RNA-seq of Gas6-stimulated Axl proficient or deficient HCC cells and VENN relations were used to identify Gas6/Axl targets. Gain- and loss-of-function studies including proteomics was employed to characterize the role of PRAME (preferentially expressed antigen in melanoma). Expression of Axl/PRAME was assessed in publicly available HCC patient datasets and in 133 HCC cases.

Results: Exploitation of well-characterized HCC models expressing Axl or devoid of Axl allowed the identification of target genes among them the cancer testis antigen PRAME. Intervention with Axl/MAPK/ERK1/2 signaling resulted in strongly reduced PRAME expression. PRAME levels associated with functional de-differentiation of HCC cells and epithelial to mesenchymal transition (EMT) augmenting 2D cell migration and 3D cell invasion. The interaction with pro-oncogenic proteins including CCAR1 suggested further tumor-promoting functions of PRAME in HCC. Moreover, PRAME showed elevated expression in Axl-stratified HCC patients which correlates with vascular invasion and lowered patient survival.

Conclusion: PRAME is a *bona fide* target of Gas6/Axl/ERK signaling linked to EMT and cancer cell invasion in HCC.

P01-15
Inhibiting endoplasmic reticulum stress enhances the effect of doxorubicin by affecting lipid metabolism in hepatocellular carcinoma

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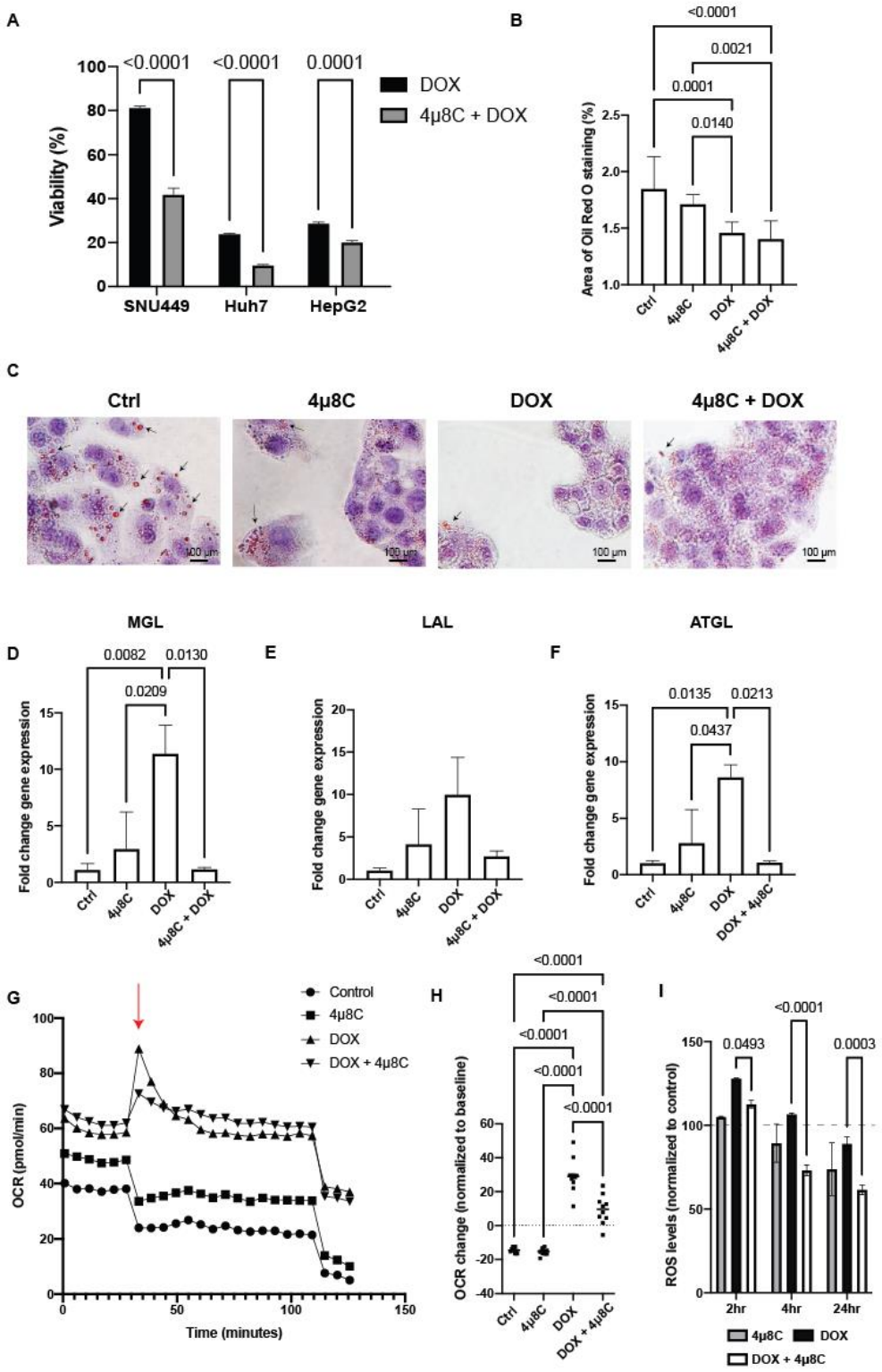
Background and Aims:
Hepatocellular carcinoma (HCC) is the most common form of liver cancer, accounting for 85% to 90% of all liver cancer cases. HCC is characterized by a high resistance to chemotherapeutic agents, while a potential contributing factor to this resistance, is the activation of the endoplasmic reticulum (ER) stress pathways. This is a cellular stress mechanism that becomes activated when the cell´s need for protein synthesis exceeds the ER´s capacity to ensure accurate protein folding, and has been implicated in creating drug-resistance in several solid tumors. In addition, there is a close link between activation of ER-stress pathways and alterations in lipid metabolism, since the ER is the main site of lipid production. The aim of this study is to establish how inhibiting IRE1α influences lipid metabolism and how this affects the response to doxorubicin (DOX).

Method:
A chemically induced mouse model for HCC was used and mice were treated twice per week with the ER-stress inhibitor 4μ8C and/or DOX for 3 weeks after the occurrence of tumors. Liver samples were taken for histological, molecular biology and lipidomics analyses. Cells were exposed *in vitro* to 4μ8C and/or DOX to further assess the effect on viability, lipid metabolism and oxygen consumption rate.

Results:
Mice with HCC experienced a statistically significant weight loss compared to healthy mice. This was further exacerbated after DOX-treatment, while co-treatment with 4μ8C restored weight levels to nearly those of healthy mice. All treatments significantly decreased the number of tumors, with the strongest reduction in the 4μ8C+DOX combination treatment. SNU99, Huh7 and HepG2 cells lines were exposed to DOX alone or in combination with 4μ8C for 24 hours and the combination with 4μ8C potentiated the cytotoxic effect of DOX in all three cell lines. Oil-Red-O staining confirmed that all treatments reduced lipid droplets in HepG2-cells with the strongest reduction in the combination group. The combination treatment also decreased the levels of MGL, LAL and ATGL, suggesting a lower level of reducing agents and a lower anabolic tone for all biomolecules. Moreover, the increase in the oxygen consumption rate (OCR) after treatment with DOX in HepG2-cells was significantly reduced when DOX was given in combination with 4μ8C.

Conclusion:
By using an *in vivo* model known for its similarity to human HCC, we show that using an ER-stress inhibitor can potentiate the cytotoxic effect of DOX, by lowering the tumor cell's anabolic tone of biomolecules, including lipids and thereby, deprive tumor cells from their energy reserves. The long-term impact of our study could open the possibility of ER-stress inhibitors as adjuvant treatments for HCC-patients, as they could enhance the efficacy of DOX, for instance during TACE-treatment.

Figure: Treatment with DOX and 4μ8C alters lipid metabolism of liver cells *in vitro*.



P01-17
Poor compliance in the surveillance for Hepatocellular carcinoma (HCC) in a real world setting

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Background and Aims: HCC is the 5TH most common cancer worldwide and the 3RD leading cause of cancer-related death. Chronic viral, alcoholic or nonalcoholic fatty liver disease (either cirrhotic or advanced fibrotic) represent the main risk factors. Surveillance of patients with chronic liver disease is a key factor in early diagnosis and treatment. The aim of the study is to assess compliance in monitoring for HCC according to international guidelines, as well as the effects of non-compliance on early diagnosis and treatment

Method: Consecutive patients with HCC who presented at our Liver Unit between Jan 2017-Dec 2021 were included in the analysis. Demographic, clinical data, imaging and laboratory information were recorded. Inadequate monitoring for HCC was defined as, less than one visit to the Hepatology department per year and failure to perform regular ultrasound, every 6 months in patients with cirrhosis and in patients with chronic hepatitis B who met the criteria of the PAGE-B score. Staging of HCC was conducted according to BCLC staging system.

Results: The study included 68 patients (73% male, mean age 67±10 years). 48/68 (72%) had diagnosed liver disease before the onset of HCC. 52/68 (77%) patients had cirrhosis (Child Pugh A, 67%). 57% had a history of decompensation with ascites (38%), variceal bleeding (25%) and encephalopathy (16%). Hepatitis B and C were the main cause of liver disease (62%), followed by alcoholic liver disease (18%) and non-alcoholic fatty liver disease (4.4%).

The diagnosis of HCC was based on imaging, while a biopsy of the lesion was performed in 1/5 of the patients (21%). 42% had an AFP value <10ng/ml, while 56% had an AFP value >400ng/ml. BCLC staging 0/A, B, C, D at diagnosis was 43%, 19%, 27.6%, 10.4% respectively. Locoregional therapy received 15%, systemic 22%, a combination of locoregional and/or systemic therapy 43% of the patients.

Inadequate HCC surveillance was recorded in 65% (32/48) of patients with previously diagnosed liver disease. Poor monitoring was found to be associated with more advanced disease according to BCLC staging (stage C and D vs 0/A, B: 21 vs 12, p=0.012), with larger lesion size at diagnosis (7.7±4.8 vs 5, 2±7 cm, p=0.005), with multifocal HCC (p=0.07), and with secondary foci (none of the patients under observation had metastases at diagnosis). Patients with alcoholic liver disease (9/11) and HCV infection (17/19) had poor follow-up compared with other causes of liver disease. 23/68 (34%) patients died of liver disease after a median period of 11 (1-60) months and 10% from a non-hepatic cause.

Conclusion: More than a half of our patients with HCC especially those with HCV and alcohol abuse had poor compliance to the scheduled follow-up strategy and presented with more advanced HCC-BCLC stage. Our data highlight the need to raise the awareness of physicians and patients for the implementation of monitoring programs for the early diagnosis of HCC.

P01-20
In silico electrophysiological study reveals Atezolizumab, an important therapeutic agent for liver cancer causes cardiac toxicity by inhibiting sodium current

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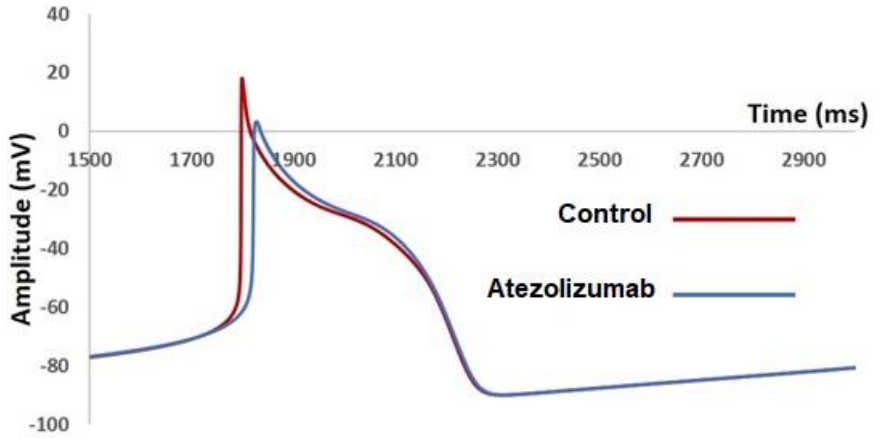
Background and Aims: Atezolizumab is a used as an important therapeutic agent to treat urothelial carcinoma, small cell lung cancer, and hepatocellular carcinoma. Accumulated clinical and experimental evidence support that Atezolizumab causes sinus bradycardia, which influences the quality of life. Cardiotoxicity due to the use of Atezolizumab is still under clinical investigation. The purpose of this study is to clarify the propensity of Atezolizumab concentration in modulating cardiac electrophysiological properties.

Method: The electrophysiological set up of the sinoatrial node (SAN) comprises the inward rectifier ion channels, sodium channels, potassium channels, calcium channels, and calcium diffusion mechanisms. Concentration-dependent Atezolizumab (0.1 µmol/L to 10 µmol/L) profile for 200 ms is induced to alter the conductance of voltage-gated sodium ion channel (Nav1.5) and then incorporated into the SA node electrophysiology. Both current-clamp and voltage-clamp protocols are applied to record the electrophysiological activities.

Results: After injecting a current stimulus (Istim) of varying magnitude (0.1-0.10 nA) and duration (10-50 ms), action potentials (AP) are reproduced by the SA node. The modulating effects of Atezolizumab concentration on the SA node's electrophysiological properties are investigated in two folds. First, we reproduced the current-voltage (I-V) curve profile of the Nav1.5 ion channel with respect to multiple doses of Atezolizumab under the voltage clamp protocol. It showed the continuous decrease of inward current because of multiple doses of Atezolizumab. At the highest concentrations (10 µmol/L), the peak of the inward current reduced to 26% of its' control value. The I-V curve is shifted to a 20% more positive side and the half-activation potential is increased by 28%. Then, the altered inward current is incorporated into the whole-cell model to investigate the AP firing patterns. For 10 µmol/L of Atezolizumab, the repolarization phase of AP was prolonged and the frequency of the firing pattern was reduced. Figure shows the AP for both control and Atezolizumab injection.

Conclusion: Our study suggests that Atezolizumab at a higher concentration reduces the frequency rate of the spontaneous AP firing by suppressing the Nav1.5 current. Therefore, the dosage of Atezolizumab should be controlled to avoid cardiac toxicity. Further clinical trials are essential to analyze its' subcellular mechanisms.

Figure:


40
20
0
-20
-40
-60
-80
-100
Amplitude (mV)
1500
1700
1900
2100
2300
2500
2700
2900
Time (ms)
Control
Atezolizumab

P02-01
A novel hepatitis B virus genome integration hotspot in DEPDC5 intron-10 predicts unfavourable prognosis for hepatocellular carcinoma patients

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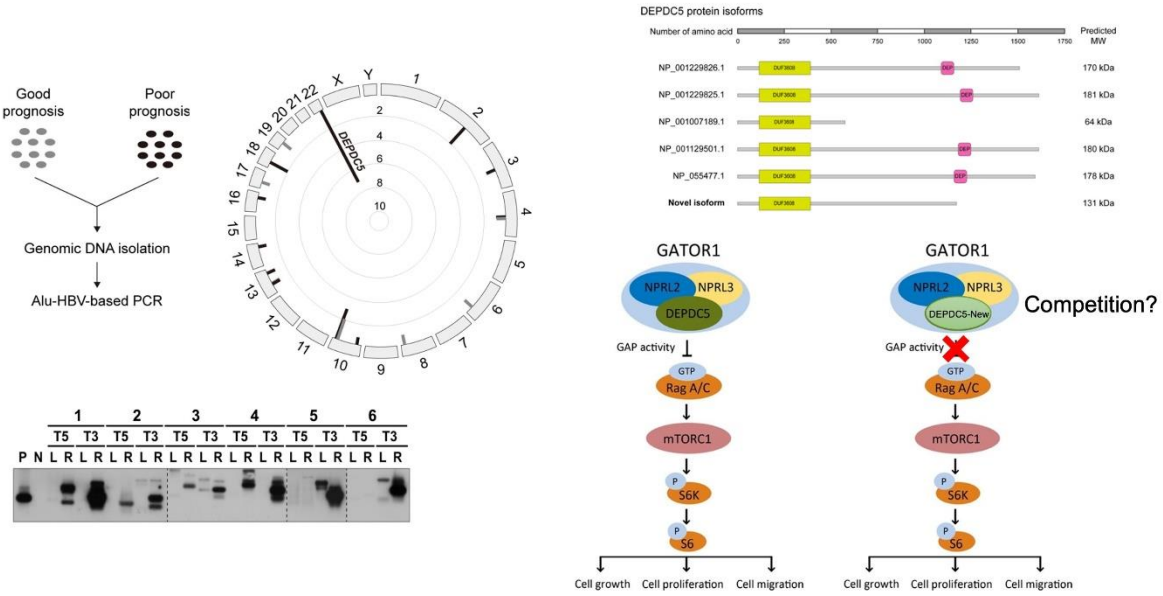
Background and Aims: Integration of the hepatitis B virus (HBV) genome into host genomic DNA (gDNA) is common in patients with HBV-associated hepatocellular carcinoma (HCC). Several HBV integration sites have previously been identified as prognostic predictors. However, the prevalence of each integration in HCC remains low, largely due to the nature of random integration. Here, we studied HBV integrations in non-cancerous and cancerous HCC tissues.

Method: A total of three groups were retrospectively included. First, twenty patients (10 favourable, 10 unfavourable) were used as an exploration cohort to identify novel integrants using Alu-HBV polymerase chain reaction (PCR). A second cohort containing 210 tissue pairs and a third set containing 300 blood samples were used to examine the presence of HBV-*DEPDC5* and HBV-*TERT* integrants by Southern blotting. Kaplan-Meier plots were used to assess whether the integrants were prognosis-relevant. Reverse transcription-PCR (RT-PCR) was used to identify novel *DEPDC5* transcripts and to assess their abundance. Western blot and immunohistochemical staining were used to study the expression level of DEPDC5 in HBV-related HCC. Cell-based assays were used to understand the role of the novel DEPDC5 variant in HCC.

Results: A novel high-frequency integration of HBV into *DEPDC5* gene was found in the paraneoplastic or neoplastic tissues, which was associated with HCC postoperative prognosis. Additionally, HBV-*DEPDC5* was also present in plasma-derived cell-free DNAs (cfDNAs). Those with the HBV-*DEPDC5* integrant were correlated with an unfavourable prognosis. RT-PCR identified a novel shorter variant of *DEPDC5* with a large exon-skipped region, resulting in the loss of the DEP domain in the protein product. This small isoform of *DEPDC5* was found predominantly in HBV-related HCC tissues. Overexpression of this novel DEPDC5 variant promoted HCC cell proliferation and migration by activating the mammalian target of rapamycin (mTOR) signaling pathway.

Conclusion: HBV-*DEPDC5* integrant in tissue-derived genomic DNA or in circulating cfDNAs can be used as a prognostic biomarker. A novel isoform of short DEPDC5 serves as an oncogene by activating mTOR signaling pathway in HBV-related HCC.

Figure: Identification of a novel HBV integration hot spot in DEPDE5 gene (left), which led to preferential generation of a novel short DEPDE5 isoform (right upper), resulting in a new oncogenic pathway (right lower).



P02-02
Mining the “dark proteome” to identify novel biomarkers in hepatocellular carcinoma

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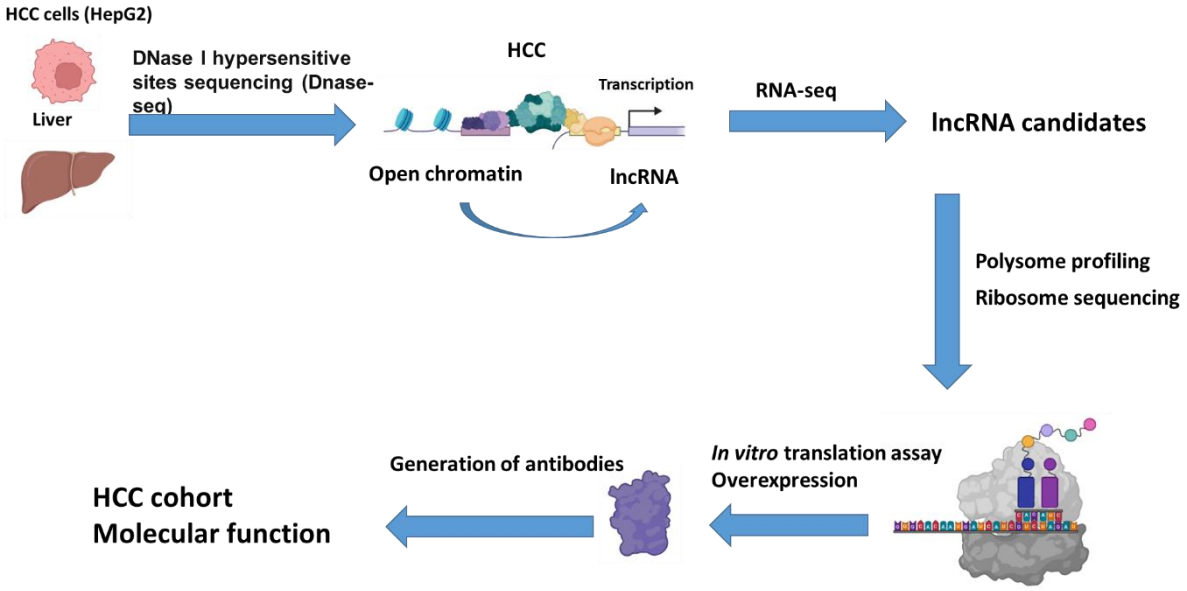
Background and Aims: A key remaining frontier in our understanding of biological systems is the “dark proteome”—that is, proteins encoded by long noncoding RNAs (lncRNAs) where the molecular function is largely unknown. The key aspect of this work is that it combines big data mining and pathology to explore the “dark proteome” in hepatocellular carcinoma (HCC), a highly aggressive cancer with limited therapeutic options. Considering that there are very few accurate molecular biomarkers for HCC detection, understanding function for the entities involved and their potential role in diagnosis and patient stratification will bring substantial impact in HCC therapy.

Method: We performed DNase I hypersensitive sites sequencing and RNA-seq for HepG2 cells and control liver to map open chromatin regions that associate with transcription of HCC-specific lncRNAs. Polysome profiling and ribosome sequencing were applied to identify lncRNAs that are translated. Peptide specific antibodies were generated for C20orf204-189AA and Linc013026-68AA, two of HCC-specific lncRNA-encoded small proteins. Immunohistochemical staining and biochemical assays were performed to examine the expression of these novel proteins in a HCC cohort and the underlying molecular functions.

Results: We successfully generated specific antibodies for C20orf204-189AA and Linc013026-68AA, two of HCC-specific lncRNA-encoded small proteins. Both proteins promote cancer cell proliferation. At the molecular level, we show that C20orf204-189AA participates in ribosomal RNA transcription, while Linc013026-68AA may be phosphorylated by Epidermal Growth Factor Receptor (EGFR) and extracellular signal-regulated kinase (ERK). Remarkably, C20orf204-189AA protein was detected in 70% of primary HCCs but not in but not in control livers, suggesting that HCC-specific lncRNA-encoded proteins may represent a novel class of biomarkers and HCC targets.

Conclusion: Our finding provides important insights into molecular functions of small proteins originating from “dark proteome” and their potential value as biomarkers or drug targets in HCC.

Figure: Strategy to identify HCC-specific lncRNA encoded small proteins (created with BioRender.com)



P02-06-YI
Novel platinum-based chemotherapeutic agents halt cholangiocarcinoma progression through the induction of inter-strand DNA breaks, preventing DNA repair mechanisms

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Background and Aims: Cholangiocarcinoma (CCA) comprises a heterogeneous group of biliary malignant tumors characterized by dismal prognosis. The first-line treatment for advanced CCA [cisplatin (CisPt) and gemcitabine] is considered palliative due to the high chemoresistance of this cancer, barely impacting on patients' overall survival. Here, we aimed to design, synthesize and study a new generation of platinum (Pt)-derived chemotherapeutic drugs that produce inter-strand DNA breaks (vs classical single-strand breaks induced by CisPt and related compounds) and thus, prevent the development of DNA repair mechanisms in cancer cells.

Method: Ten Pt-derivatives (Aurki-Pt#s) were designed and synthesized. Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM) were used to characterize the binding of Aurki-Pt#s to DNA. The antitumoral effect of the two best candidates (Aurki-Pt#1 and #2) was evaluated by measuring the viability of human CCA cells (EGI-1 and HUCCT1), newly generated CisPt-resistant EGI-1 CCA cells, normal human cholangiocytes (NHC) and cancer-associated fibroblasts (CAFs). The DNA damage induced by Aurki-Pt#1 and #2 was assessed using the comet assay. To ascertain the internalization mechanism of Aurki-Pt#1 and #2, substrate competition studies through flow cytometry and accumulation studies using HPLC-MS/MS were carried out. Finally, the effect of Aurki-Pt#1 and #2 was also tested *in vivo* on a subcutaneous xenograft model of CCA.

Results: Aurki-Pt#s induced inter-strand DNA breaks, and the subsequent DNA fragmentation, contrary to CisPt. Aurki-Pt#1 and #2 significantly reduced CCA cell viability. Both compounds triggered increased DNA damage in CCA cells when compared to CisPt, augmenting the reactive oxygen species levels and being more effective when inducing apoptosis *in vitro*. Additionally, Aurki-Pt#1 and #2 decreased the proliferative capacity of those CCA cells that survived. Importantly, Aurki-Pt#1 and #2 also promoted cell death in CisPt-resistant CCA cells. Moreover, Aurki-Pt#1 and Aurki-Pt#2 caused CCA spheroid shrinkage. On the contrary, Aurki-Pt#1 and #2 did not induce a lethal effect in NHC in culture, but promoted cell cycle arrest. Besides, Aurki-Pt#1 and Aurki-Pt#2 had an impact on the survival of CAFs. Aurki-Pt#1 and #2 were transported into cells through OCT1, OCT3, CTR1 and OATP1A2, which did

not transport CisPt. Finally, Aurki-Pt markedly hampered tumor growth on a subcutaneous xenograft model of CCA in comparison with CisPt or vehicle control.

Conclusion: This new generation of Pt-derived chemotherapeutic drugs selectively diminishes CCA cell viability through the induction of inter-strand DNA breaks, and has an impact on its tumor microenvironment, representing a promising therapeutic tool for naïve or CisPt-resistant CCA tumors.

P02-07-YI
Modelling hepatocellular carcinoma in precision-cut liver slices for therapeutic screening and as a precision medicine tool

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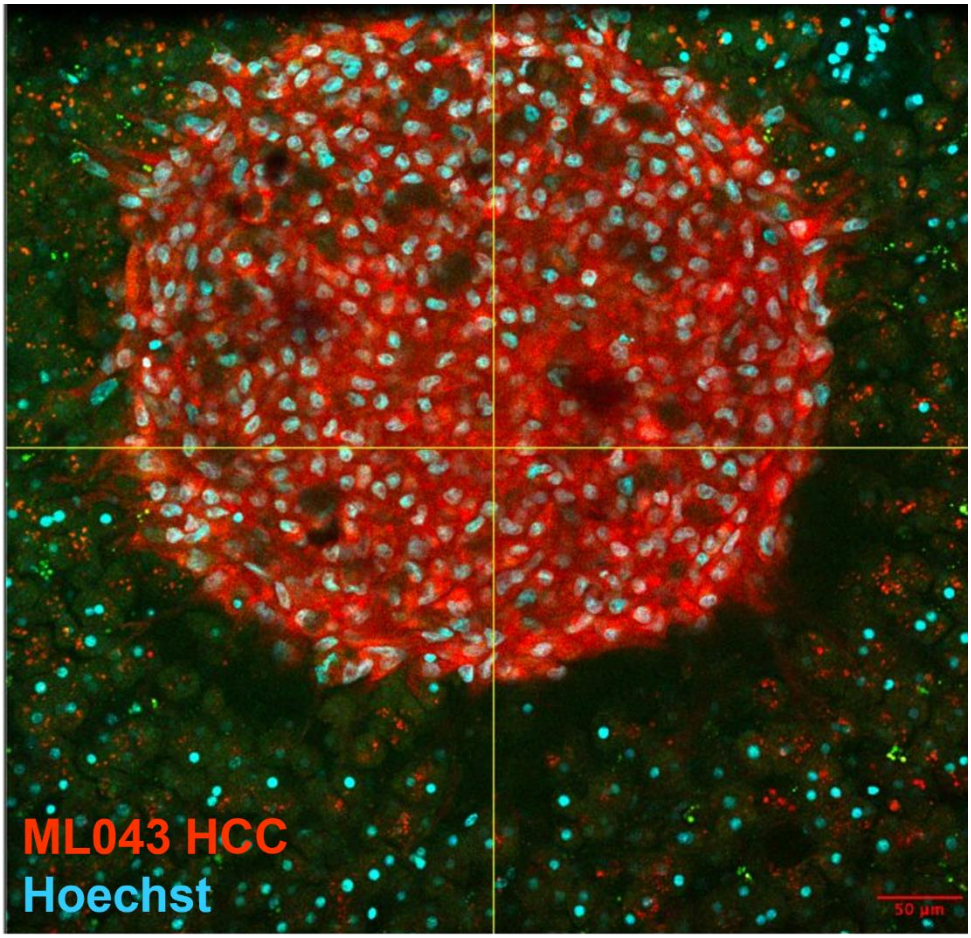
Background and Aims: Liver cancer is one of the most common causes of cancer-related death worldwide, and hepatocellular carcinoma (HCC) accounts for approximately 90% of cases. Recent therapeutic advances extend overall survival by a few months, but only for a minority of patients. Realistic HCC models are necessary in order to bridge the gap between preclinical research and *in situ* human disease, and provide valuable insight into disease pathogenesis and drug discovery. Here we describe the development of two HCC models in precision-cut liver slices (PCLS): a murine precision-cut tumour slice (PCTS) model utilising the Hep-53.4 cell line, and a spheroid-engrafted human PCLS model.

Method: Hep-53.4 PCTSs were generated from orthotopic tumours 21 days after an intrahepatic injection of 1 million cells in C57BL/6 mice. PCTSs were subsequently cultured in the presence or absence of the receptor tyrosine kinase (RTK) inhibitors sorafenib or lenvatinib, or with an inhibitor of the immune checkpoint PD-1. An *ex vivo* human HCC model was developed by engrafting spheroids generated from HuH7 cells that express a secreted luciferase, onto human PCLSs. The spheroid-engrafted PCLS were then cultured in the presence or absence of the RTK inhibitors sorafenib, lenvatinib or regorafenib. Primary HCC cell lines derived from patient biopsies were expanded and lentiviral-transduced to express mCherry, and spheroids generated from these cells were also implanted onto human PCLSs. All tissue was cultured in a bioreactor system capable of maintaining the viability of the tissue for 7 days.

Results: Treatment of Hep-53.4 PCTSs with the RTK inhibitors sorafenib and lenvatinib results in decreased proliferation (Ki-67) and increased apoptosis (active caspase-3). Immunohistochemical characterisation of the PCTSs determined that they maintain a rich immune profile in culture, and treatment with anti-PD-1 immunotherapy results in significantly higher CD3 T-cell numbers, as well as increased HCC apoptosis. In relation to the human spheroid-PCLS model, complete invasion of the spheroids into the PCLSs was confirmed via multiphoton imaging. Measurement of luciferase secreted following treatment with RTK inhibitors indicated a significant and dose-dependent reduction in cancer growth, whilst the PCLSs remained viable. Primary HCC spheroids implanted onto human PCLS also displayed complete engraftment and invasion into the tissue (Figure 1).

Conclusion: The two models described potentially provide unique tools for discovery biology and precision medicine, where therapies can be tested on both PCTSs and patient-derived HCC cells in the context of the tumour microenvironment.

Figure:


ML043 HCC
Hoechst
50 µm

P02-09
Chimeric antigen receptor-engineered memory-like natural killer cells: a novel therapy for hepatocellular carcinoma

Rosalba Biondo[1], Indrani Nayak[2], Sophie Cramer[3], Melissa Quadflieg[3], Congcong Zhang[3], Nina Möker[3], Jayajit Das[2], Salim Khakoo[1]

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Background and Aims: Hepatocellular carcinoma (HCC) is one of the most common type of primary liver cancer, with high death rates and increasing recurrence. Current treatments have been ineffective and great effort has been put into improving HCC therapies, focussing on immunotherapy. Natural killer (NK) cells have been a rapidly developing approach for cancer immunotherapy due to their innate cytotoxic potential and anti-tumor functions. Hence, the aim of this study is to develop an NK-based therapy for HCC. We investigate the anti-tumor response of memory-like NK (ML-NK) cells alone, and when engineered with glypican-3 (GPC3) specific chimeric antigen receptor (CAR), which further increases cytotoxicity. ML-NK cells are generated by a short time pre-activation with cytokines, causing increased cell proliferation and enhanced anti-tumor functions upon a second stimulation. To enhance this protocol, we are implementing in-silico modelling to optimise conditions for NK cell proliferation and cytotoxicity.

Methods: NK cells were purified from peripheral blood mononuclear cells (PBMC) derived from whole blood of healthy donors and cultured for 16 days with cytokines to elicit activation. Fresh media and cytokines were replenished every two-three days, and expansion rate was monitored through cell count. Phenotype and cytotoxic potential of ML-NK cells were examined by flow cytometry at day 10 of culture. A subset of ML-NK cells was engineered to express GPC3-CAR 2 days after purification. Cytotoxic potential of CAR-ML-NK cells was tested against PLC and HepG2 cell lines. NK cell response to cytokine stimulation was predicted via a linear regression model.

Results: Short exposure to IL-21 increases expansion of ML-NK cells, when used in combination with IL-12+15+18. This is seen at day 7 of culture with 10-fold expansion increase, robustly sustained by day 16, reaching 70-fold expansion. Cytokine activation to induce ML-NK cells and CAR engineering, have an additive effect on the anti-tumor activity of NK cells. CAR-ML-NK cells, show greater cytotoxic effect against the highly GPC3-positive cell line HepG2, compared to non-transduced ML-NK. This is also seen with the relatively resistant cell line PLC, which expresses low, but not undetectable, levels of GPC3. CAR-ML-NK cells are phenotypically identified by high expression of NKG2D, NKG2C, CD16, NKG2A, and low expression of NKp44 and CD57. In-silico modelling suggests a donor specific cytokine combination to boost NK cell proliferation and cytotoxicity and quantifies the contribution of STAT molecules.

Conclusions: Improving NK cell culture protocol is crucial for NK-based therapies. This study shows that IL-21 plays key roles into NK cell biology, enhancing memory-like properties of cytokine-activated NK cells. Cytotoxicity is further augmented by addition of GPC3-specific CAR, offering a promising venue for HCC therapy.

P02-10-YI
miR-21-5p promotes NASH-related hepatocarcinogenesis

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Background and Aims: The mechanisms governing the progression of non-alcoholic fatty liver disease (NAFLD) towards steatohepatitis (NASH) and hepatocellular carcinoma (HCC) remain elusive. We have previously shown that NAFLD development is inhibited in Mir21 knockout (KO) mice treated with obeticholic acid. Here, we evaluated the role of hsa-miRNA-21-5p in NASH-related hepatocarcinogenesis.

Method: Hepatic hsa-microRNA(miR)-21-5p expression was evaluated in 2 cohorts of patients with biopsy-proven NAFLD (n=199) or HCC (n=366 HCC and n=11 NAFLD-HCC). Serum/liver metabolomic profiles were correlated with hsa-miR-21-5p in NAFLD obese patients. Wild-type (WT) and Mir21 KO mice were fed a choline-deficient, amino acid-defined (CDAA) diet for 32 and 66 weeks to induce NASH and NASH-HCC, respectively.

Results: In obese individuals, hsa-miR-21-5p expression increased with NAFLD severity and associated with a hepatic lipotoxic profile. CDAA-fed WT mice displayed increased hepatic mmu-miR-21-5p levels and progressively developed NASH and fibrosis, with livers presenting macroscopically-discernible pre-neoplastic nodules, hyperplastic foci and deregulated cancer-related pathways. Mir21 KO mice exhibited peroxisome-proliferator activated receptor alpha (PPARalpha) activation, augmented mitochondrial activity, reduced liver injury and NAS below the threshold for NASH diagnosis, with the pro-inflammatory/fibrogenic milieu reversing to baseline levels. In parallel, Mir21 KO mice displayed reduced number of pre-neoplastic nodules, hepatocyte proliferation and activation of oncogenic signaling, being protected from NASH-associated carcinogenesis. The hsa-miRNA-21-5p/PPARalpha pathway was similarly deregulated in patients with HCC or NASH-related HCC, correlating with HCC markers and worse prognosis.

Conclusion: hsa-miR-21-5p is a key inducer of whole-spectrum NAFLD progression, from simple steatosis to NASH and NASH-associated carcinogenesis. Inhibition of hsa-miR-21-5p, leading to a pro-metabolomic profile, might constitute an appealing therapeutic approach to ameliorate NASH and prevent progression towards HCC (EXPL/MED-OUT/1317/2021).

P02-13
Glycosylated 4-methylumbelliferone reduces the expressions of cancer stem cells in hepatocellular carcinoma

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Background and Aims: The inhibition of hyaluronic acid (HA) may block the growth of various cancers, including hepatocellular carcinoma (HCC). The 4-methylumbelliferone (4-MU), an HA enzymatic inhibitor, was shown to reduce hepatic fibrosis, inflammation, and the presence of cancer stem cells (CSC). Recently, we demonstrated that a glycosylated 4-MU, namely 4-MUR, with the addition of rhamnose residue in 4-MU, constituted a better strategy to target HCC cells while sparing normal liver cells due to the interaction with the rhamnose-specific asialoglycoprotein receptor in HCC cells. This study aimed to investigate the comparison between 4MUR and 4MU to the profile of the CSC profiles of HCC.

Method: The profile of CSC markers in HCC cell lines JHH6 and Huh7 upon treatment of 4-MU and 4-MUR was evaluated using flow cytometer and real-time PCR for counting of the cells and gene expression, respectively. The cytotoxicity of the compounds was assessed by MTT assay. Immortalized hepatocytes cell line IHH was used as control.

Results: 4-MUR was toxic only in HCC cell lines and not in immortalized hepatocytes. 4-MUR treatment better reduced the number of cells expressing CSC markers EpCAM and CD13/ANPEP compared to 4-MU in both HCC cell lines. Reduction of CD133/Prom-1 was also noticed in Huh7 which expresses this marker. Flow cytometric data was then compared with that in gene expression, showing concordant results. Interestingly, the 4-MUR did not reduce the expressions of both CD44, a receptor of HA, and CD90/THY-1 mRNA in IHH cells.

Conclusion: 4-MUR capacity to reduce the expressions of CSCs in HCC cells was comparable, if not better, to those of 4-MU. Importantly, 4-MUR was selective to target only HCC cells while being less efficient in normal hepatocytes.

P02-14
Cellular interrogation of changes to anti-tumour immunity in Hepatocellular Carcinoma following treatment with combined atezolizumab and bevacizumab using human Precision-Cut Tumour Slices

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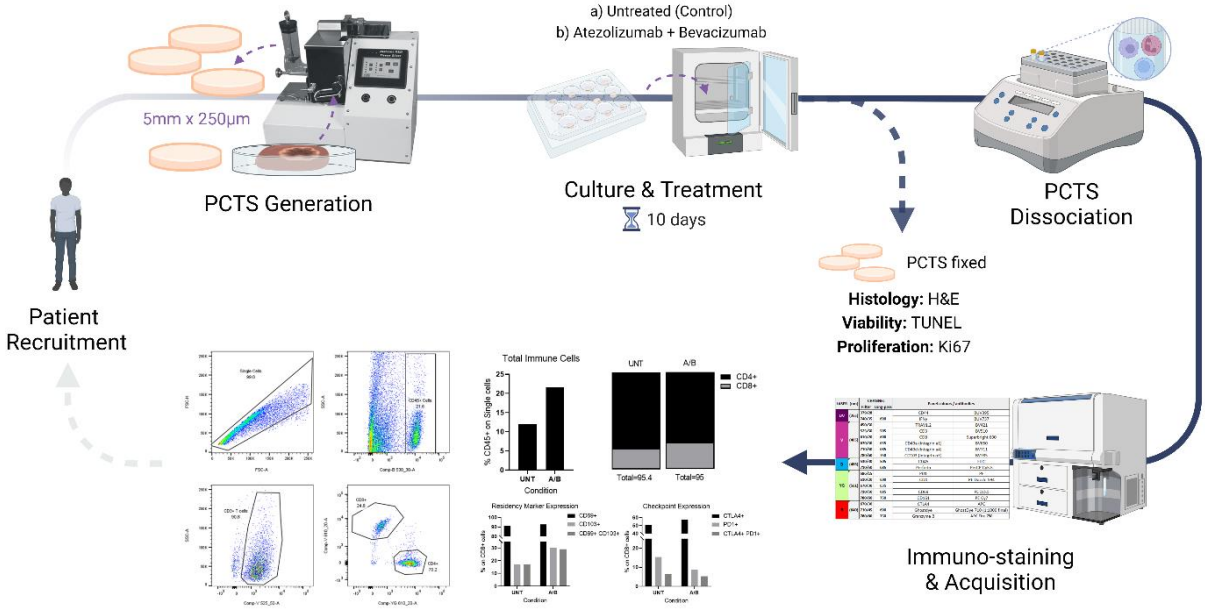
Background and Aims: Atezolizumab (anti-PD-L1) plus bevacizumab (anti-VEGF) has become the standard of care in patients with unresectable hepatocellular carcinoma (HCC). Improved clinical outcome has been associated with intra-tumoral T effector cell density, as a marker of pre-existing immunity (Zhu *et al.*, 2023). However, functional and cellular changes occurring within the tumour immune microenvironment (TIME), particularly to intra-tumoral $CD8^+$ T cells, are still unclear. Previously we have described the use of Precision-Cut Tumour Slices (PCTS) generated from human primary and secondary liver cancers as a patient-specific immunocompetent *ex-vivo* disease model, which captures the complex heterogeneity of the tumour and TIME, including infiltrating/resident immune populations and checkpoint receptor/ligand expression. In this proof-of-concept study, we demonstrate the utility of HCC PCTS for studying the patient specific intra-tumoral immune response to treatment with atezolizumab/bevacizumab.

Method: PCTS generated from resected human tumours (n=2) were cultured in the presence or absence of atezolizumab/bevacizumab ex-vivo treatment for 10 days. Untreated and treated PCTS were subsequently dissociated into single-cell suspensions and the intra-tumoral immune T cell compartments were interrogated and compared using flow-cytometry.

Results: Treatment with atezolizumab/bevacizumab [1] increased the $CD8^+$ to $CD4^+$ratio, [2] upregulated markers of $CD3^+$ T cell residency: CD103 (~1.9 fold) and CD49d (~1.8 fold), [3] raised proportions of $CD8^+$ $CD69^+$ $CD103^+$ resident-memory T cells (T_{RM}), [4] reduced $CD8^+$ T cell expression of PD-1 expression by > 2-fold and [5] noticeable increase in perforin degranulation in both $CD8^+$ and $CD4^+$ T cell. Collectively these results suggest treatment with atezolizumab/bevacizumab may activate anti-tumour functions by resident CD8+ T cells and facilitate the acquisition of a resident-memory phenotype, which has been previously associated with improved prognosis in HCC (CJ Lim et al., 2019).

Conclusion: These results demonstrate the utility of HCC PCTS for studying the effects of immunotherapeutic strategies on intra-tumoral immunity at a cellular level.

Figure:


5mm x 250µm
PCTS Generation
a) Untreated (Control)
b) Atezolizumab + Bevacizumab
Culture & Treatment
10 days
PCTS Dissociation
PCTS fixed
Histology: H&E
Viability: TUNEL
Proliferation: Ki67
Patient Recruitment
Total Immune Cells
% CD45+ on Single cells
UNT
A/B
Condition
CD4+
CD8+
Total=95.4
Total=95
Residency Marker Expression
Checkpoint Expression
CTLA4+
PD1+
Immuno-staining & Acquisition

P02-20
The role of cytochrome c oxidase subunit 4 in the adaptation to hypoxia in hepatocellular carcinoma cells

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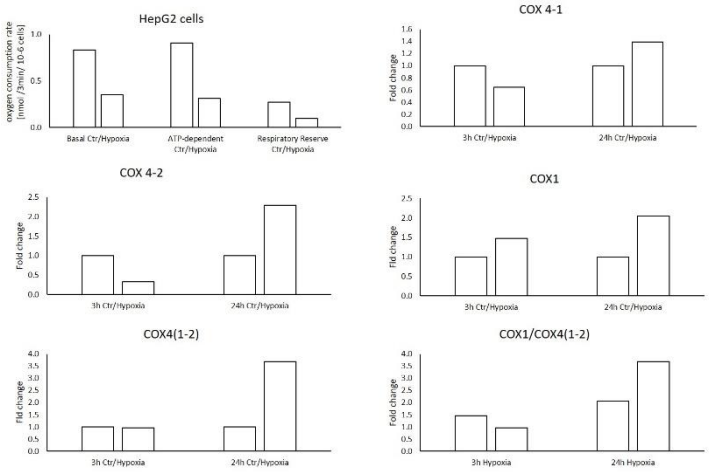
Background and Aims: The tumor microenvironment (TEM) is characterized by hypoxic conditions, and the importance of the hypoxic microenvironment in the development of hepatocellular carcinoma (HCC) and reduced therapeutic efficacy has been established. Cytochrome-c-oxidase (COX) is the last electron acceptor of the respiratory in the process of oxidative phosphorylation (OXPHOS). Nuclear-encoded COX subunit 4 (COX4) is associated with the mitochondrial subunit COX1 and plays an important role in the function, assembly, and regulation of COX. In this study, we investigated the adaptive response of HCC cells to a chemically induced hypoxia, and assessed the role of cellular respiration and COX4 in this response.

Method: We chemically induced acute (3 and 24 h) and chronic hypoxia (15 days) with $CoCl_2$ in HepG2 and HepaRG. Experiments in a hypoxic chamber containing 1% O_2 were also performed. Respiration was measured by a polarographic approach and reported as oxygen consumption rate (OCR). We then measured the expression levels and the activity of COX4 to evaluate hypoxic changes associated with dysfunction in cellular respiration and COX4/COX1 ratio, as well as promotion of fermentation metabolism.

Results: We found that cellular respiration, i.e., basal, ATP-dependent, and respiratory reserves, was reduced under hypoxic conditions compared to control normoxic cells. After 15 days of chronic exposure to $CoCl_2$, cell clones emerged and cellular respiration became less dependent on ATP, although this effect appeared to be reversible once the cells were placed under normoxic conditions. Furthermore, we found that the expression of two subunits of COX 4 (1 and 2) decreased after 3 hours of hypoxia (fold change 0.6 and 0.3, respectively), whereas the expression of the same genes increased after 24 hours (fold change 1.4 and 2.3, respectively) in HepG2 cells. Finally, the *COX4/COX1* ratio increased (fold change 3.7) in favor of *COX4* expression after 24 hours of hypoxia, suggesting that the nuclear-encoded subunit *COX4* contributes to hypoxic conditions.

Conclusion: These results suggest a role for the nuclear-encoded subunit COX4 in hypoxic TEM. COX4 is upregulated in HepG2 cells, possibly related to the uncharacterized activation of HIF-1α. Our findings support a role for the COX4 enzyme in the hypoxia-sensing pathway of energy metabolism in HCC cells.

Figure:


HepG2 cells
oxygen consumption rate [nmol / 3min/ 10-6 cells]
Basal Ctr/Hypoxia
ATP-dependent Ctr/Hypoxia
Respiratory Reserve Ctr/Hypoxia
COX 4-1
Fold change
3h Ctr/Hypoxia
24h Ctr/Hypoxia
COX 4-2
COX1
COX4(1-2)
COX1/COX4(1-2)
3h Hypoxia
24h Hypoxia

P03-01
Myeloid cell-derived HMGB1 protects from the development of hepatocellular carcinoma

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Background and Aims: HMGB1 is a non-histone chromatin-associated protein involved in the pathogenesis of chronic liver disease. HMGB1 is expressed in myeloid cells, including conventional dendritic cells (cDC) and tumor-associated macrophages (TAMs), which play a major role in the tumor microenvironment. However, whether intracellular myeloid cell derived HMGB1 is involved in HCC is unknown. Our aim was to investigate whether intracellular HMGB1 drives cDC maturation towards LAMP3$^+$ DCs and decreases immunosuppressive TAMs in the tumor; hence, allowing effective cytotoxic CD8$^+$ T cell responses to reduce HCC.

Methods: we analyzed publicly available scRNA-seq datasets from human HCC for the expression of HMGB1 in all subsets of DCs and TAMs in HCC tumor and non-tumor tissue and in hepatic draining lymph nodes (dLNs). We generated mice with conditional ablation or overexpression of *Hmgb1* in myeloid cells (*Hmgb1*$^{\Delta Mye}$ and *Hmgb1*$^{KI\ Mye}$). To induce HCC, 14-days-old male mice were injected i.p. with diethylnitrosamine (DEN) and were sacrificed at 5, 6, 8 and 10 months.

Results: mature LAMP3$^+$ DCs increase in human and mouse HCC tumor tissue and hepatic dLNs. TAMs, with low expression of HMGB1, are proangiogenic and immunosuppressive, and increase in human and mouse HCC tumor tissue. *Hmgb1*$^{KI\ Mye}$ mice are fully protected from HCC, whereas control mice develop HCC after 8 months and *Hmgb1*$^{\Delta Mye}$ mice start developing HCC at 5 months. Macroscopic analysis and H&E staining of the livers from *Hmgb1*$^{\Delta Mye}$ mice shows more tumors and higher tumor volume compared to control and *Hmgb1*$^{KI\ Mye}$ mice. Immunohistochemistry of HCC tumor sections reveals that *Hmgb1*$^{\Delta Mye}$ mice have increased infiltration of TAMs. Analysis of immune cell populations by flow cytometry shows that *Hmgb1*$^{\Delta Mye}$ mice have less mature LAMP3$^+$ DCs in liver and hepatic dLNs compared to control and *Hmgb1*$^{KI\ Mye}$ mice, suggesting less CD8$^+$ T cell activation. In addition, there is enhanced CD8$^+$ T cell apoptosis in the HCC tumor tissue from *Hmgb1*$^{\Delta Mye}$ mice.

Conclusion: ablation of myeloid derived HMGB1 accelerates HCC development in mice. Therefore, increasing HMGB1 expression in specific myeloid cell subsets (cDCs and TAMs) could be a therapeutic approach to protect from HCC.

P03-02-YI
MAP17 promotes an epithelial-mesenchymal-amoeboid transition in hepatocellular carcinoma by switching one-carbon metabolism

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Background and Aims: Epithelial-mesenchymal transition (EMT), a key process during embryonic development, promotes cell migration and resistance to apoptosis during tumour invasion and metastasis. In hepatocellular carcinoma (HCC) an amoeboid behaviour tends to increase the aggressiveness and metastatic capacity of epithelial tumours.
MAP17 is a 17kDa membrane protein expressed during embryogenesis, absent in most adult organs. The presence of MAP17 correlates with an inflammatory environment, hypoxia and increased reactive oxygen species (ROS). MAP17 has been identified in several types of cancer, including HCC. Modulation of EMT and amoeboid behaviour via MAP17 offers an attractive approach to prevent metastasis.

Method: Two HCC patient cohorts were used to characterise MAP17 levels. *In vitro*, expression of MAP17 was measured in mesenchymal and epithelial hepatoma cells, and its levels were modulated to study cell proliferation, drug resistance, mitochondrial dynamics, metabolic rewiring, and proteome homeostasis. *In vivo*, the role of MAP17 in the metastatic capacity was evaluated using orthotopic HCC mouse models.

Results: A positive correlation between MAP17 and mesenchymal markers, RAC/RHO family genes and markers of amoeboid movement was established in 751 HCC patients by *in silico* studies and by mRNA expression analysis in 246 HCC patients. MAP17 overexpression in 3D epithelial cell experiments let to the formation of rosette invadopodia, proinvasive structures with high metastatic capacity.

MAP17 overexpression *in vitro* induced a reprogramming of energy metabolism in hepatoma cells with epithelial phenotype, increasing mitochondrial dynamics and Warburg effect-mediated lactic acidosis, which support a tumour microenvironment conducive to cancer cell proliferation. ROS generation was increased as a protective mechanism to avoid apoptotic and senescence processes. Rewiring of the one-carbon metabolic pathway was identified, proving an accelerated metabolism of the cell. There was a faster methionine degradation fuelling the folate cycle, which is the source of purines and pyrimidines, supporting a higher proliferative state. Thus, MAP17 could be involved in the methionine cycle, specially affecting the folate cycle.
Accordingly, overexpression of MAP17 in PLC/PRF/5 cells led to the formation of multiple tumour foci when orthotopically implanted in the mouse liver.
MAP17 silencing in hepatoma cells with mesenchymal phenotype led to the opposite results, regressing the tumour phenotype and slowing down the cell metabolism and proliferation.

Conclusion: Modulation of MAP17 in epithelial and mesenchymal HCC cells leads to the reprogramming of the transitional genes that define each phenotype. Our findings have identified the metastatic potential of MAP17 in liver cancer, as it triggers the mesenchymal phenotype and amoeboid behaviour in HCC.

P03-03-YI
Targeting the E2F/MCM axis in cholangiocarcinoma halts disease progression in experimental models by rewiring lipid metabolism

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Background and Aims: Cholangiocarcinoma (CCA) comprises a heterogeneous group of biliary cancers with dismal prognosis. E2F1 and E2F2, transcription factors that regulate cell cycle and metabolism, are upregulated in metabolic associated fatty liver disease, which is a risk factor of CCA. E2F1/2 drive MAFLD-related hepatocellular carcinoma (HCC) development, sustaining a pro-carcinogenic lipid-rich environment. Minichromosome maintenance (MCM) proteins, helicases involved in DNA replication and cell cycle, are recognized targets of E2Fs that have been linked to different cancers. Thus, the aims were to evaluate the involvement of the E2F/MCM axis in CCA, and to investigate the potential therapeutic regulatory value of E2F/MCM axis in the rewiring of cancer lipid metabolism.

Method: *Akt1* and *Yap* or *Taz* were overexpressed in the liver of wild type (WT) or *E2f1*$^{-/-}$ mice as models of CCA. CCA cancer associated fibroblasts (CAFs) were isolated from patients. Triglyceride (TG) concentration was measured in liver samples of the mouse models of CCA and in cell lines *in vitro*. Cell viability, proliferation, spheroid growth and fatty acid oxidation (FAO) rate were measured in EGI1 and HUCCT1 CCA cell lines in the presence or absence of ciprofloxacin (CPX), an antibiotic that inhibits the MCM2-7 helicase activity. The effect on CCA cell viability, proliferation and spheroid growth of an inhibitor of E2F activity (HLM006474) was also tested. Data from human CCA tumours from the TCGA-CHOL cohort were analysed.

Results: Expression of E2F1 and E2F2 was upregulated in human CCA tumours, patient-derived CAFs, and in cellular and mouse models of CCA compared to controls; consequently, expression levels of MCM2-7 were also elevated, and correlated positively with E2F1/2 expression. Overexpression of *Akt1* and *Yap*, or *Akt1* and *Taz*, in *E2f1*$^{-/-}$ mice resulted in significantly reduced tumour development compared to WT mice. The upregulation of E2F1/2 and MCM2-7 in CCA cells and tumours in mice was accompanied by increased TG content. Inhibition of MCM activity in human CCA cells with CPX induced a dose-dependent decrease in tumour cell viability, proliferation and spheroid growth. CPX also reduced the TG content and the FAO of CCA cells (EGI1) *in vitro*, which we had previously shown to be highly dependent on FAO for proliferation, and incubation with HLM006474 also decreased cell viability,

proliferation and spheroid size. Combination of CPX and HLM006474 induced a higher reduction of CCA cell viability *in vitro* than CPX or HLM006474 alone, suggesting that E2F activity promotes CCA progression not only by modulating MCM expression.

Conclusion: The E2F/MCM axis is upregulated in CCA and is required for tumour cell survival and proliferation, potentially by affecting, among other mechanisms, the increased TG content, FAO rate and the tumour microenvironment, arising as a novel target for therapy in this cancer.

P03-05
Centrosome amplification is attributed to glutamine metabolism in the developments of liver fibrosis and liver tumors

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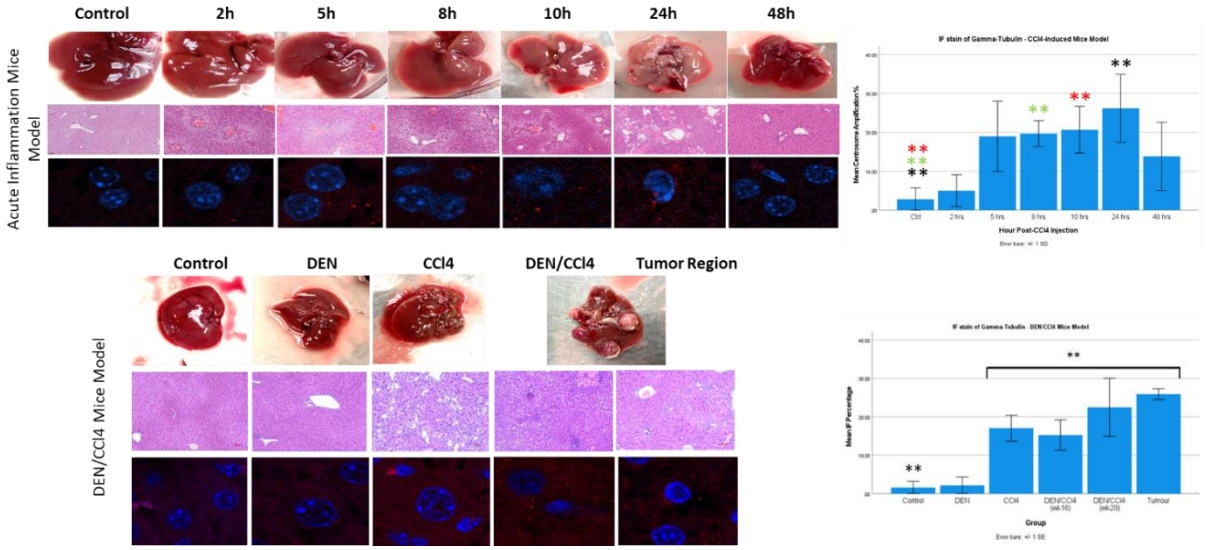
Background and Aims: Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer that continued to increase worldwide in Asia. It is attributed to liver fibrosis, glutaminolysis, and centrosome amplification (CA). Patients who suffered from chronic liver diseases usually develop liver fibrosis by glutaminolysis. CA also plays a role in liver tumorigenesis and metastasis, but its association with liver fibrosis and glutaminolysis remains unclear. This is a novel study investigating the relationship between CA and glutaminolysis during the developments of liver fibrosis and HCC. We aim to identify if CA is attributed to glutaminolysis.

Method: In this study, we investigated the relationship between glutaminolysis and CA in the development of liver fibrosis in CCl_4-induced acute inflammation mice model and HCC in DEN/CCl_4-induced chronic mice model, respectively.

Results: In our mice models, CA was observed in mice livers that developed liver fibrosis or tumors. We found that overexpressed gamma-tubulin in both mice models have shown that CA was triggered by glutamine metabolism. Gene expression levels of glutamine transporters, glutamine-related cytokines, and glutamine-fuelled centrosome regulators were also significantly upregulated in both mice models.

Conclusion: This is a novel study investigating the relationship between CA and glutaminolysis. Our studies have shown that CA is attributed to glutaminolysis during the developments of liver fibrosis and HCC alike.

Figure:


Control
2h
5h
8h
10h
24h
48h
Acute Inflammation Mice Model
IF stain of Gamma-Tubulin - CCl4-Induced Mice Model
Hour Post-CCl4 Injection
Control
DEN
CCl4
DEN/CCl4
Tumor Region
DEN/CCl4 Mice Model
Group

P03-06
Proteomic profile matching predicts treatment response in advanced hepatocellular carcinoma for precision medecine

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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 advanced HCC can only be 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 prescribed in first-line treatment. However, the response rate to this combination therapy does not exceed 20%, according to studies. 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. The calculation of the distance of the test patient from the known responses provides a score allowing us to predict the response to the treatment.

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. Using machine learning, we are able to predict patient response of HCC patients to these two treatments used respectively in first and second line.

Conclusion: Through this study we demonstrate the tumor proteomic profiling relevance in precision medicine for advanced HCC patient's management. This result highlights the resource capacity of liver biopsies and the added value to include mass spectrometry-based proteomic analysis into the HCC process for patient management.

P03-07
Identification and experimental validation of druggable epigenetic targets in hepatoblastoma

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Background and Aims: Hepatoblastoma (HB) is the most frequent childhood liver cancer. Surgical resection is the mainstay treatment which frequently is preceded by neoadjuvant chemotherapy (cisplatin or doxorubicin). However, patients with aggressive tumors have limited therapeutic options; therefore, a better understanding of HB pathogenesis is needed to improve treatment. HB have a very low mutational burden; however, epigenetic alterations are increasingly recognized. We aimed to identify epigenetic regulators consistently dysregulated in HB and to evaluate the therapeutic efficacy of their targeting in clinically relevant models.

Method: We performed a comprehensive transcriptomic analysis of 180 epigenetic genes. Data from fetal, pediatric, adult, peritumoral (n=72) and tumoral (n=91) tissues were integrated. Selected epigenetic drugs were tested in HB cells. The most relevant epigenetic target identified was validated in primary HB cells, HB organoids, a PDX model, and a genetic mouse model. Transcriptomic, proteomic and metabolomic mechanistic analyses were implemented.

Results: Altered expression of genes regulating DNA methylation and histones modifications was consistently observed in association with molecular and clinical features of poor prognosis. The histone methyl-transferase G9a was markedly upregulated in tumors with epigenetic and transcriptomic traits of increased malignancy. Pharmacological targeting of G9a significantly inhibited HB cells, organoids and PDX's growth. Development of HB induced by oncogenic forms of β-catenin and YAP1 was ablated in mice with hepatocyte-specific deletion of *G9a*. We observed that HB undergo significant transcriptional rewiring in genes involved in amino acids metabolism and ribosomal biogenesis. G9a inhibition counteracted these pro-tumorigenic adaptations. Mechanistically, G9a targeting potently repressed the expression of c-MYC and ATF4, master regulators of HB metabolic reprogramming.

Conclusion: HB display a profound dysregulation of the epigenetic machinery. Among them, G9a is upregulated in association with tumor aggressiveness. Pharmacological targeting of G9a potently quells HB growth. Inhibition of G9a abrogates the c-MYC and ATF4-mediated metabolic reprogramming that supports cancer cell survival. Pharmacological targeting of key epigenetic effectors exposes metabolic vulnerabilities that can be leveraged to improve the treatment of these patients.

P03-08-YI
Spheroids derived from aggressive hepatocellular carcinoma (HCC) or intrahepatic cholangiocarcinoma (iCCA) share similar behaviour after proangiogenic stimulation

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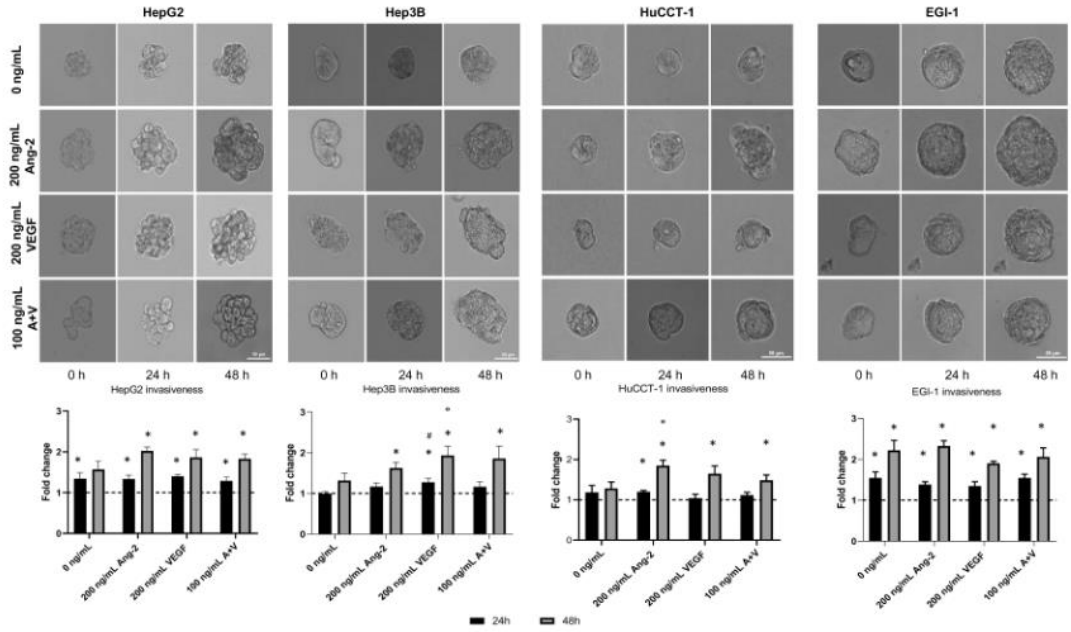
Background and Aims: Aggressive HCCs overexpressing Angiopoietin-2 (Ang-2) (a protein linked with angiogenesis, proliferation, and epithelial-mesenchymal transition [EMT]), share 95% of upregulated genes and similar severe prognosis with iCCA proliferative subgroup. We analysed the effect of proangiogenic stimuli on in vitro 3D models, to uncover possible common ways of response between aggressive HCCs and iCCA.

Method: We generated spheroids from 4 cell lines: HepG2 and Hep3B from HCC, HuCCT-1 (from iCCA) and EGI-1 (from extrahepatic CCA, eCCA). Spheroids were stimulated with either 200 ng/mL Ang-2 or VEGF, or with 100 ng/mL of Ang-2 + VEGF. We analysed migration at 3, 24 and 48 hours (h) and invasion in Matrigel at 0, 24, and 48 h from stimulation. We evaluated EMT markers expression: E-cadherin (E-cad), N-cadherin (N-cad), Vimentin (Vim) by Western blot (WB) and immunofluorescence (IF). Unstimulated spheroids served as negative controls.

Results: Proangiogenic stimuli increased migration vs. controls in HepG2, Hep3B and HuCCT-1 spheroids, but not in EGI-1. Specifically, Ang-2 alone boosted HepG2 and HuCCT-1 spheroids migration, while VEGF alone or plus Ang-2 determined the highest increment in Hep3B at 48 h. In Matrigel, HepG2 spheroids, including controls, showed increased invasiveness at 24 h while at 48 h only those stimulated (all 3 treatments) displayed significantly higher size than at baseline. In Hep3B a significant increase of size was obtained with VEGF while in HuCCT-1 with Ang-2 at 48 h. EGI-1 spheroids showed a steady gain in size both at 24 and 48 h, independently from stimulation.
In stimulated HepG2 spheroids, a clear decrease of E-cad and an increase of N-cad and Vim levels was evidenced in WB (already at 3 h for the cadherins and at 48 h for Vim). In Hep3B and HuCCT-1 spheroids a time-dependent change of these markers was observed: in Hep3B E-cad decreased and N-Cad and Vim increased with VEGF alone or plus Ang-2, in HuCCT-1 only Vim increased under Ang-2 stimulation. In EGI-1 all EMT markers did not change in any condition, further reinforcing the differences between iCCA and eCCA. The IF analysis revealed a marked decrease in E-cad and increase in N-cad expression at the migration front, both in HCCs and in iCCA spheroids suggesting the presence of a cadherin switch in the peripheral cells of the spheroids.

Conclusion: The increase of migration and invasiveness in HepG2, Hep3B and HuCCT-1 spheroids and the EMT data after Ang-2 ± VEGF clearly show a close but not identical response to proangiogenic stimuli, indicating strong resemblances between HCC (especially those derived from Hep3B) and HuCCT-1 spheroids in response to proangiogenic stimulation while reiterating the strong dissimilarity with eCCA-derived spheroids (Supported by AIRC, ID:24858).

Figure: Invasion of spheroids after proangiogenic stimuli (p<.05 vs. baseline; # vs. ctl at 24 h; ° vs. ctl at 48h).



P03-10-YI
RIPK3 impacts tumor burden and liver immunophenotype in toxic/dietary models of hepatocellular carcinoma

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Background and Aims: Receptor-interacting protein kinase 3 (RIPK3) is a well-established key executor of necroptosis and promotes NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome activation and pyroptosis. We have previously shown that RIPK3-dependent signalling is triggered under chronic liver injury in humans, while its inhibition arrested disease progression *in vivo*. Still, the precise role of RIPK3-dependent signalling in hepatocarcinogenesis and immune modulation remains elusive. Here, we aimed to investigate the impact of blocking RIPK3 in liver carcinogenesis and tumour microenvironment (TME).

Method: Two-week-old male C57BL/6 wild-type mice (WT) or *Ripk3*-deficient (*Ripk3*$^{-/-}$) pups were injected with diethylnitrosamine (DEN; i.p. 25 mg/kg), followed by feeding with a choline deficient – high fat diet (CDHFD) or a standard diet (SD) from 4 to 42-weeks-old. In parallel studies, mice were fed from 4 to 57-weeks-old with CDHFD. The liver was removed and macroscopic tumours were counted and measured for phenotypic characterization. Gene expression analyses were performed to evaluate markers of inflammation, fibrosis and infiltrated immune cells. Liver samples were freshly processed for immunophenotyping by flow cytometry.

Results: Macroscopically discernible tumours were only detected in DEN-treated mice, and tumour burden was exacerbated in mice injected with DEN and fed with CDHFD. Ablation of *Ripk3* abrogated tumour frequency in both models and reduced tumour size in the DEN model. *Ripk3* deficiency halted hepatic macrophage infiltration and inflammation in both DEN-treated and CDHFD-fed mice. Despite the lack of effect on T cell infiltration, both programmed death-ligand 1 (*Pd-l1*) and *Pd-1*, major players in suppressing the adaptive arm of the immune system, were globally abrogated in samples from *Ripk3*$^{-/-}$ mice, compared with WT counterparts. This was accompanied by a general decrease in the expression of *Nlrp3* and its downstream effectors in pyroptosis, caspase-1 and interleukin-1beta.

Conclusion: *Ripk3* deficiency reduced hepatic tumour burden in both toxic and dietary models of hepatocellular carcinoma. This was accompanied by changes in infiltration of macrophages and in their inflammatory profile. Notably, although not impacting T cell infiltration, *Ripk3* deficiency likely dampens T cell exhaustion in TME. In particular, our results indicate that *Ripk3* deletion impacts the PD-L1/PD-1 axis, likely by impairing NLRP3 inflammasome activation, which may improve patient response to immunotherapy.
(Support by 2021.07666.bd and PTDC/MED-FAR/3492/2021, FCT; LCF/PR/HR21/52410028, La Caixa Foundation)

P03-13
Therapeutic potential of engineered oncolytic virus 3020-VV13 in a pre-clinical model of hepatocellular carcinoma

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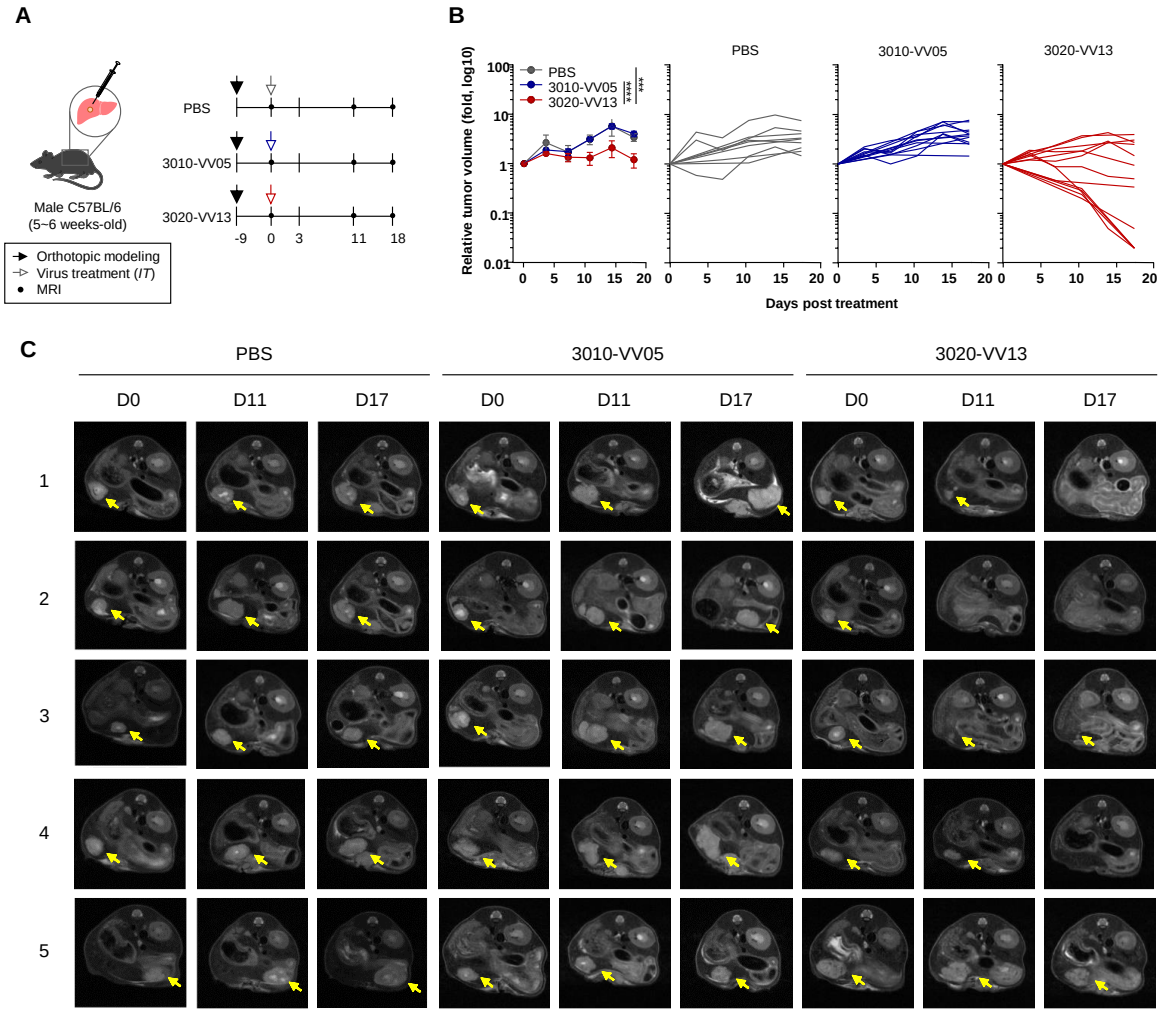
Background and Aims: Despite advances in treatment options, the prognosis for patients with hepatocellular carcinoma (HCC) remains poor. To improve outcomes for these patients, there has been growing interest in the use of oncolytic virus for HCC. Oncolytic virus exhibits both high selectivity and immune stimulatory properties towards cancer cells. In this study, we aim to evaluate the efficacy of an engineered oncolytic virus, 3020-VV13, in a pre-clinical orthotopic model embodying the tumor microenvironment of HCC.

Method: We generated a genetically modified vaccinia virus by introducing hyaluronidase PH-20, interleukin-12, and soluble PD-1 to improve its efficacy in the tumor microenvironment. The efficacy of the modified oncolytic virus, 3020-VV13, was evaluated in a syngeneic orthotopic mouse model of HCC, using real-time magnetic resonance imaging. Gene expression profiling was performed using NanoString nCounter PanCancer IO 360 Panel.

Results: Treatment with 3020-VV13 significantly inhibited tumor growth *in vivo*. while the *in vitro* proliferation showed similar anti-proliferative activities compared with the control virus. Moreover, the change of intrahepatic cytokines was accompanied by dynamic immune responses in the orthotopic tumors. The anti-tumoral efficacy of 3020-VV13 was related to the expressed transgenes and altered immune-related gene profiles.

Conclusion: Our study demonstrates the potential of 3020-VV13 as a promising therapeutic agent for HCC. The virus showed improved anti-tumoral efficacy compared to the parental virus and was able to modulate the immune responses effectively. Further development of 3020-VV13 may provide a new option for HCC therapy.

Figure:


A
PBS
3010-VV05
3020-VV13
-9
0
3
11
18
Male C57BL/6
(5~6 weeks-old)
Orthotopic modeling
Virus treatment (IT)
MRI
B
PBS
3010-VV05
3020-VV13
Relative tumor volume (fold, log10)
100
10
1
0.1
0.01
****
***
0
5
10
15
20
Days post treatment
C
PBS
3010-VV05
3020-VV13
D0
D11
D17
1
2
3
4
5

P03-15
Promotion of differentiation reduces hepatocellular carcinoma cell growth by boosting respiration

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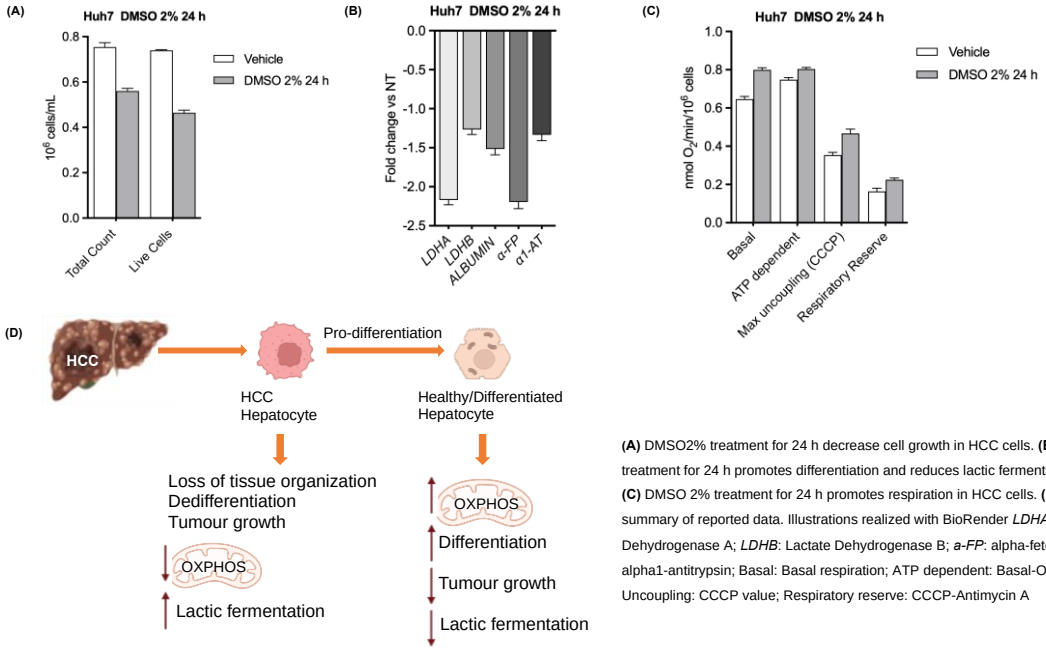
Background and Aims: Hepatocellular carcinoma (HCC) is becoming one of the "big killers" tumors, especially in Western countries. Conventional therapeutic and pharmacological approaches do not provide an adequate answer to this emergency. Hence, novel biological understandings are needed in the effort to improve the current treatment options for HCC. Our aim was to demonstrate a functional link between cellular differentiation, respiration, and growth, where an increase in differentiation should lead to a decrease in HCC growth with concomitant enhancement of cellular respiration/oxidative phosphorylation.

Method: We treated the Huh7 cell line with 2% DMSO as a pro-differentiation agent and analyzed cell growth and differentiation by detecting the expression of pro-differentiation genes. Then, we performed a “respiration fingerprint” by using a polarographic approach, which allows to measure total oxygen consumption and to characterize respiration, by using oxidative phosphorylation selective inhibitors.

Results: Our results demonstrate that the promotion of differentiation is associated with a decrease in HCC cell growth and with an increase in respiration, meaning a stimulation of the efficiency of oxidative phosphorylation. These results point to a functional link between differentiation, respiration, and cell growth.

Conclusion: Taken together, our findings suggest that promoting differentiation reduces HCC cell growth through enhanced respiration and decreased lactic acid fermentation. This provides a functional correlation between HCC cell growth, differentiation, and oxidative phosphorylation/fermentation. Future research will extend these investigations to other pro-differentiating agents and will explore the opportunity to exploit the promotion of differentiation as a therapeutic opportunity. Finally, we will investigate the link between differentiation, oxidative metabolism, and drug resistance.

Figure:



(**A**) DMSO2% treatment for 24 h decrease cell growth in HCC cells. (**B**) DMSO 2% treatment for 24 h promotes differentiation and reduces lactic fermentation in HCC cells. (**C**) DMSO 2% treatment for 24 h promotes respiration in HCC cells. (**D**) Graphical summary of reported data. Illustrations realized with BioRender *LDHA*: Lactate Dehydrogenase A; *LDHB*: Lactate Dehydrogenase B; *a-FP*: alpha-fetoprotein; *a1-AT*: alpha1-antitrypsin; Basal: Basal respiration; ATP dependent: Basal-Oligomycin; Max. Uncoupling: CCCP value; Respiratory reserve: CCCP-Antimycin A

P03-16
Lysosomotropic PIP5K inhibitors sensitize hepatic cancer to reactive oxygen species by inhibiting proliferative and adaptive pathways

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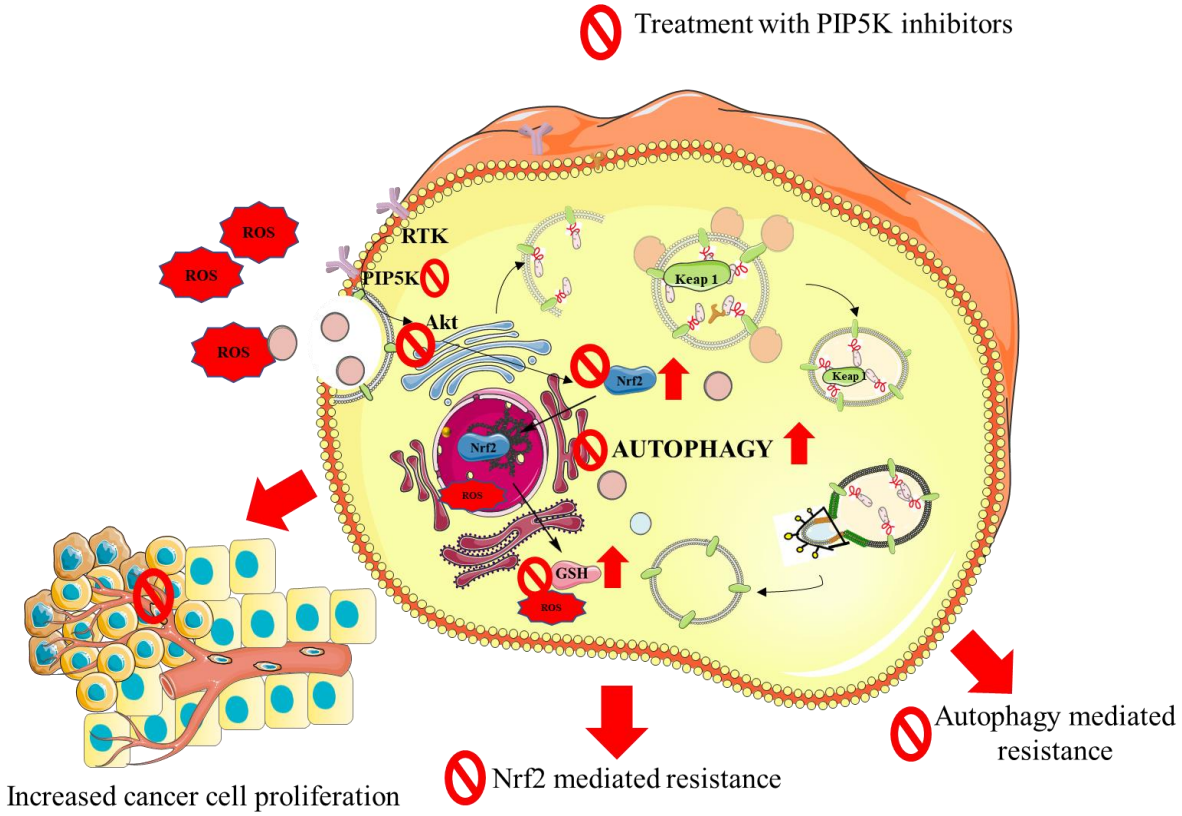
Background and Aims: The complex interplay between ROS-molecular signaling governs proliferation, adaptation, and death. To date, how cancer cells modulate PIP5K levels to control proliferative and adaptive signaling is not known.

Method: Firstly, we investigated the protein expression of PIP5K isoforms, Beclin-1 (autophagy marker), and Nrf2 (antioxidant master regulator) in 36 HCC patients and immortalized cells viz PRF5, SNU-387, Skhep-1 and HepG2. To understand the ROS-mediated effect on PIP5K, autophagy, and antioxidants, HCC cells were exposed to H2O2. Further, the effect on cell viability, mitochondrial superoxide, lysosome turnover, expression of PIP5K isoforms, autophagy and antioxidant enzymes through MTT, MitoSOX, and lysotracker was evaluated. The effect of PIP5K inhibition on cancer cells sensitization was investigated with novel investigational molecules NG-TZ-17 and IITZ01, and the finding was confirmed with standard PIP5K1A inhibitor ISA-2011B. Also, the Autophagy inhibition of NG-TZ-17 and IITZ01 was examined with respect to autophagy inhibitor Chloroquine and NRF2 inhibitor ML-385. In vivo GFP-HepG2-induced hepatic cancer model in SCID mice was developed for exploring therapeutic efficacy. In HCC Mice, 50 mg/kg NG-TZ-17 and IITZ01 and compared with 60 mg/kg Sorafenib administered orally for seven days.

Results: We observed a positive correlation between PIP5K isoforms, Beclin-1 and Nrf2 in hepatocellular carcinoma liver tissue and cell lines. Cytotoxicity and Protein expression showed a concentration-dependant starvation-induced proliferation, and resisted cell death by increasing PIP5K isoforms, Nrf2, HO-1, and SOD2 levels. At cytotoxic concentration resulted in autophagic cell death as indicated by the decrease in PIP5K isoforms, Akt, Nrf2, HO-1, and SOD2 expression however, an increase in SRC and Beclin-1was observed. The inhibition of PIP5K with lysosomotropic NG-TZ-17, IITZ01, and ISA-2011B (standard) sensitized HCC cells showed proliferative, autophagy and Nrf2 pathway downregulation compared to HCC cells exposed to mild stress. We observed that the PIP5K inhibitors (both) were superior to Chloroquine and ML385. In vivo, we observed that 50 mg/kg NG-TZ-17 ($p<0.001$) and IITZ01 ($p<0.001$) reduced the tumor burden in the HepG2-xenograft SCID mice model ($p<0.001$ compared to tumor control). The treatment was equipotent to standard sorafenib 60 mg/kg ($p<0.001$ vs tumor control).

Conclusion: HCC clinical and in vitro data showed that PIP5K isoforms involved the switching of cancer cells from adaptive to proliferative state and vice-versa in response to ROS levels. PIP5K inhibitors sensitized cancer cells to mild ROS. Thus, targeting PIP5K will overcome the limitations of standard RTK, autophagy and Nrf2 inhibitors. This study also confers the therapeutic efficacy of novel PIP5K inhibitors viz NG-TZ-17 and IITZ01 in Hepatic cancer.

Figure:


Treatment with PIP5K inhibitors
ROS
ROS
ROS
RTK
PIP5K
Akt
Keap 1
Keap 1
Nrf2
Nrf2
AUTOPHAGY
ROS
GSH
ROS
Increased cancer cell proliferation
Nrf2 mediated resistance
Autophagy mediated resistance

P03-17
Mangiferin & Resveratrol; novel nutraceuticals regulating CD8+ cell cytotoxicity and pivotal immunoregulatory pathways in hepatocellular carcinoma

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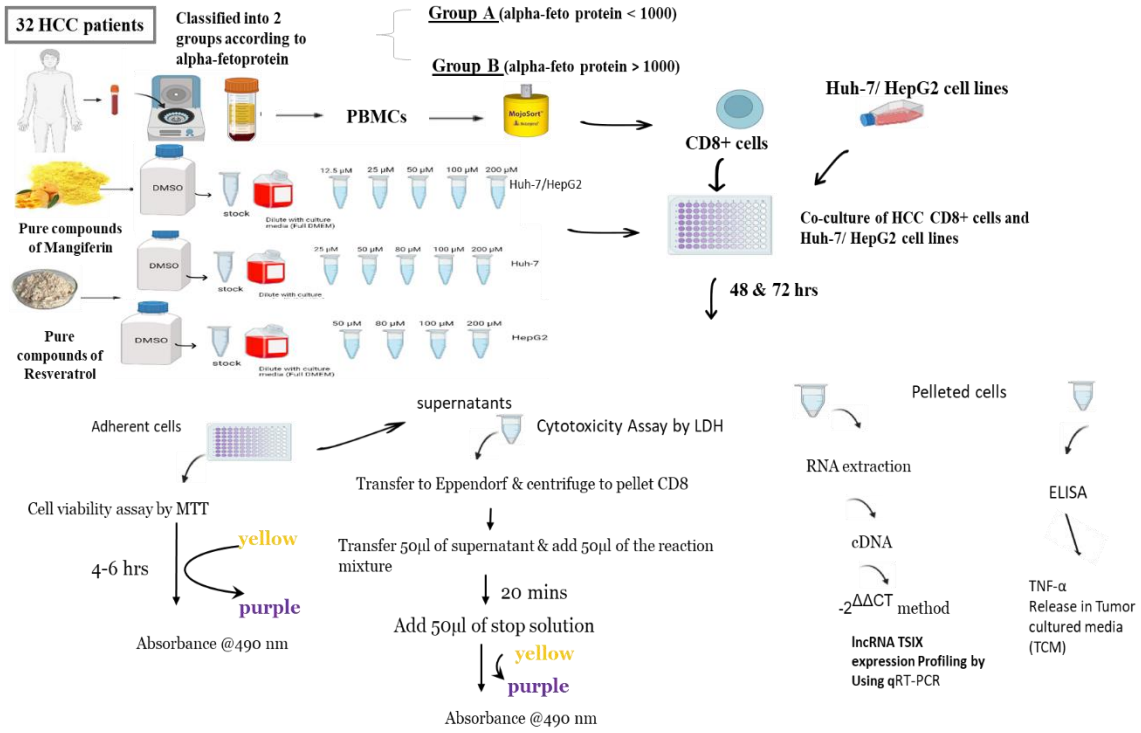
Background and Aims: Hepatocellular carcinoma (HCC) occurrence and progression are linked to the immune milieu in the liver. Lately, tumor-immune cells interaction has received remarkable interest, in addition to the fact that many molecular pathways connected to the biological features of tumor cells were reported to significantly influence the immune system in a mechanism known as cancer immune evasion. Consequently, immunotherapy became one of the most effective strategies of cancer therapy. Recent studies showed that the use of nutraceuticals can support immune restoration and thus, reduces the severity of HCC with minimal side effects. Mangifera indica commonly known as mango, and Vitis vinifera commonly known as Grapes specifically their active substances, Mangiferin and Resveratrol are among the most extensively investigated natural sources to improve the efficiency of anti-tumor immunity thus decreasing the disease severity and improving patients' quality of life. This study aims at investigating the immunoregulatory impact of these natural products on CD8+ cells of HCC patients.

Method: CD8+ T cytotoxic cells were isolated from blood of 32 HCC patients (classified into 2 groups based on alpha-feto protein levels). CD8+ cells were co-cultured with 2 HCC cell lines (Huh7 and HepG2) using different concentrations of both compounds. At 0, 48 and 72 hrs post treatment, MTT and LDH assays were used to measure cell viability and cytotoxicity, respectively. Release of Tumor necrosis factor-alpha (TNF-α) protein in tumor-cultured media (TCM) was quantified using ELISA. In addition to assessing the expression of a previously reported oncogenic non-coding RNA gene long non-coding RNA TSIX using Real-Time Quantitative Reverse Transcription (qRT-PCR).

Results: Mangiferin and Resveratrol showed remarkable enhancement in CD8+ cytotoxic effect and a decrease in % cellular viability post 48 Hours of treatment of both cell lines with Mangiferin doses; 12.5µM, 25µM, 50µM, 100µM, 200µM, ($P = < 0.05$) for most of the doses compared to untreated cells and Resveratrol doses; 25µM, 50µM, 80µM, 100µM, 200µM in Huh-7 cell lines and doses 50µM, 80µM, 100µM, 200µM in HepG2 cell lines ($P = < 0.05$) for most of the doses compared to untreated cells. However, the effect was reversed post 72 hours of treatment at specific doses. A decrease in TNF-α release in TCM has been observed 48 and 72 hours post treatment ($P = < 0.05, 0.001$, respectively) for most of the doses compared to untreated condition. Moreover, the gene expression of the oncogenic LncRNA TSIX has shown a significant decrease post 48 and 72 hours of treatment ($P < 0.0001$) compared to untreated cells.

Conclusion: The outcome of the study highlights the immunoregulatory role of Mangiferin and Resveratrol pure compounds in liver cancer and paves the road for their utilization as an approach to potentiate HCC immunotherapies.

Figure:


32 HCC patients
Classified into 2 groups according to alpha-fetoprotein
Group A (alpha-feto protein < 1000)
Group B (alpha-feto protein > 1000)
PBMCs
CD8+ cells
Huh-7/ HepG2 cell lines
Pure compounds of Mangiferin
DMSO
stock
Huh-7/HepG2
Huh-7
HepG2
Co-culture of HCC CD8+ cells and Huh-7/ HepG2 cell lines
48 & 72 hrs
Pure compounds of Resveratrol
Adherent cells
supernatants
Cytotoxicity Assay by LDH
Pelleted cells
RNA extraction
ELISA
Cell viability assay by MTT
4-6 hrs
yellow
purple
Absorbance @490 nm
Transfer to Eppendorf & centrifuge to pellet CD8
Transfer 50µl of supernatant & add 50µl of the reaction mixture
20 mins
Add 50µl of stop solution
yellow
purple
Absorbance @490 nm
cDNA
-2ΔΔCT method
lncRNA TSIX expression Profiling by Using qRT-PCR
TNF-α Release in Tumor cultured media (TCM)

P03-19
In vivo characterization of the impact of distinct p53 mutations in hepatocellular carcinoma

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Background and Aims: Although the tumor suppressor p53 has been extensively studied, the precise role of its mutants in oncogenesis has not yet been clarified. This partial characterization is due to the pleiotropic functions of p53, implemented through its many protein partners and a multitude of target genes. Reports indicate that oncogenic mutants of p53 are not all equivalent, sometimes presenting tissue-specific and distinct phenotypes from that induced by the loss of expression of $p53^{WT}$. This suggests the acquisition of a "Gain of function" phenotype by some mutants. It is essential to better characterize it, especially in order to define treatments adapted to inter-tumoral heterogeneity. Around 30% of hepatocellular carcinomas (HCC) are altered for Tp53, constituting a subgroup of aggressive and poorly differentiated tumors. The consequences of distinct p53 mutations in this context are not established. We have selected eight p53 mutations relevant for HCC (V157F, A159P, R175H, R248W, R248Q, R249S, R273C, R273H) in order to characterize their physiopathological effects on liver carcinogenesis, in comparison with the inactivation of p53.

Method: We performed hydrodynamic tail vein injection (HDTVi) in C57BL/6J mice to combine overexpression of the oncogene c-myc with 8 different p53 mutants. Inactivation of Trp53 with crispr/cas9 editing technique was used as control. Tumor burden were analyzed at the macroscopic level to assess tumors number and size. Tumors were characterized by qRT-PCR and immunohistochemistry (IHC). RNAseq analyses of the transcriptomic programs are ongoing. To get further insight into mechanistic aspects of mutant p53 activities, we generated tumor-derived cell lines from tumors of distinct genetic combinations.

Results: Our results show that the combinations of c-myc and $p53^{MUT}$ lead to the development of numerous hepatocellular carcinomas within 2 to 6 weeks. The analysis of tumor burden identified two highly oncogenic mutants (R175H, R249S), others with a tumor burden similar to p53 inactivation (R248W, R248Q, R273C), and three that are only slightly oncogenic (A159P, V157F, R273H) in this model.

Conclusion: These results confirm the non-equivalence of p53 mutations in the context of hepatocellular carcinoma and suggest the acquisition of a gain-of-function phenotype for certain mutants. Experiments are ongoing to validate this observation and identify the molecular mechanisms involved in this process.

P04-01-YI
Liquid biopsy protein biomarkers of cholangiocarcinoma risk, early diagnosis and survival mirroring tumor cells

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Background and Aims: Cholangiocarcinoma (CCA), heterogeneous biliary tumors with dismal prognosis, lacks accurate early-diagnostic methods, especially important for individuals at high-risk (i.e., primary sclerosing cholangitis (PSC)). Here, we searched for protein biomarkers in serum extracellular vesicles (EVs).

Method: EVs from patients with isolated PSC (n=45), concomitant PSC-CCA (n=44), PSC who developed CCA during follow-up (PSC to CCA; n=25), CCAs from non-PSC etiology (n=56), hepatocellular carcinoma (n=34) and healthy individuals (n=56) were characterized by mass-spectrometry. Diagnostic biomarkers for PSC-CCA, non-PSC CCA or CCAs regardless etiology (pan-CCAs) were defined and validated by ELISA. Their expression was evaluated in CCA tumors at single-cell level. Prognostic EV-biomarkers for CCA were investigated.

Results: High-throughput proteomics of EVs identified diagnostic biomarkers for PSC-CCA, non-PSC CCA or pan-CCA, and for the differential diagnosis of intrahepatic CCA and HCC, that were cross-validated by ELISA using total serum. Machine learning-based algorithms disclosed CRP/FIBRINOGEN/FRIL for the diagnosis of PSC-CCA (local disease (LD)) vs isolated PSC (AUC=0.947;OR=36.9), and when combined with CA19-9, overpowers CA19-9 alone. CRP/PIGR/VWF combination allowed the diagnosis of LD non-PSC CCAs vs healthy individuals (AUC=0.992;OR=387.5). Noteworthy, CRP/FRIL accurately diagnosed LD pan-CCA (AUC=0.941;OR=89.4). Levels of CRP/FIBRINOGEN/FRIL/PIGR showed predictive capacity for CCA development in PSC before clinical evidences of malignancy. Multi-organ transcriptomic analysis revealed that serum EV-biomarkers were mostly expressed in hepatobiliary tissues, and scRNA-seq and immunofluorescence analysis of CCA tumors showed their presence mainly in malignant cholangiocytes. Multivariable analysis unveiled EV-prognostic biomarkers, with COMP/GNAI2/CFAI and ACTN1/MYCT1/PF4V associated negatively or positively to patients' survival, respectively.

Conclusion: Serum EVs contain protein biomarkers for the prediction, early diagnosis and prognosis estimation of CCA detectable using total serum, representing a tumor cell-derived liquid biopsy tool for personalized medicine.

P04-03
Exportin-1 (XPO-1) as a novel target for natural killer cells in hepatocellular carcinoma

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Background and Aims: Cancer immunotherapy of hepatocellular carcinoma (HCC) is a rapidly evolving field, with Atezolizumab and Bevacizumab as the standard of care for unresectable HCC. Natural killer (NK) cells are an emerging opportunity for cancer immunotherapy mediating both direct, and antibody dependent, cellular cytotoxicity, and thus suitable for combination therapy with antibodies, such as anti-PDL1. We previously identified a novel strategy for activating NK cells through the receptor KIR2DS2 that mediated enhanced anti-HCC activity in a xenogenic model. The aim of this study was to investigate the mechanism of this activity, and to determine its clinical relevance.

Results: KIR2DS2 is a peptide:MHC receptor, therefore to identify ligands for KIR2DS2, MHC class I peptides were eluted by immunoprecitipation from HUH7:HLA-C*0102 hepatoma cells and the parental HUH7 cell line and quantified by mass spectrometry. 13,000 peptides were sequenced and only one NAPLVHATL had the canonical binding motif for KIR2DS2 binding. This peptide is derived from the nuclear export protein exportin-1 (XPO-1/crm-1), which is commonly upregulated in HCC and assorts with a poor prognosis. Binding of NAPLVHATL to HLA-C was demonstrated in MHC class I stabilization assays using the TAP-deficient cell line 721.174 and to KIR2DS2 shown using a KIR2DS2 tetramer. Molecular modelling confirmed key KIR2DS2-peptide interactions. In functional assays there was augmented killing of HUH7:C*0102 cell lines by KIR2DS2-positive NK cells as compared to KIR2DS2-negative cell lines and this killing was reduced by knockdown of exportin-1 by siRNA at all effector:target ratios tested ($p<0.001$), confirming exportin-1 as a molecular target for KIR2DS2-positive, but not KIR2DS2-negative NK cells.

To investigate the clinical relevance of our findings we queried the GDC-Pan-cancer dataset containing RNAseq data of 11,768 cancers using the Xena informatics tool (http://xena.ucsc.edu/). In the cohort of hepatocellular carcinomas (407 subjects) expression of the gene *ncr1* (which specifically marks NK cells) was associated with improved 3 and 5 year survival in tumours expressing high levels of exportin-1 ($p=0.005$ and $p=0.03$ respectively), compared to exportin-1 low tumours ($p>0.5$ for both timepoints). Similar associations were found for other NK cells associated genes including *NKG7*, *KLRB1*, *KLRD1*, *KLRK1* and *KLRF1*. Of 22 other cancers in the GDC-Pan-cancer dataset, association of *ncr1* and exportin 1 with survival was found in only one other tumour type (head and neck cancer), thus identifying exportin-1 as specific target for NK cells in HCC

Conclusion: We identify exportin-1 as providing the first described peptide:MHC class I cancer-associated ligand recognised by natural killer cells, and show its association with outcome in HCC. Exportin-1 is thus a novel target for NK cell-based immunotherapy for HCC.

Figure:

Kaplan-Meier survival analyses of the GDC-TCGA HCC dataset (407 samples) were performed for the indicated NK cell genes. HCC samples were categorized into high and low exportin-1 levels based on median expression. Shown are the p values for the curves between groups expressing high and low levels of the indicated NK cell genes, in each of the exportin-1 categories, indicating association of expression of NK cell genes with long-term survival.

NK cell gene	Exportin-1 level	1 year survival	2 year survival	3 year survival	4 year survival	5 year survival
Ncr1 (NKp46)	High	0.296	0.018	0.005	0.015	0.029
	Low	0.731	0.672	0.915	0.599	0.478
NKG7	High	0.006	0.003	0.0007	0.001	0.002
	Low	0.252	0.983	0.694	0.377	0.889
KLRB1 (CD161)	High	0.008	0.003	0.002	0.009	0.0089
	Low	0.589	0.942	0.217	0.341	0.505
KLRD1 (CD94)	High	0.068	0.026	0.005	0.015	0.032
	Low	0.646	0.986	0.544	0.368	0.223
KLRF1 (NKp80)	High	0.007	0.002	0.002	0.001	0.002
	Low	0.720	0.831	0.562	0.300	0.109
KLRK1 (NKG2D)	High	0.030	0.006	0.002	0.003	0.005
	Low	0.814	0.669	0.560	0.366	0.328

P04-06-YI
Scavenger receptor MARCO is associated with an immunosuppressive microenvironment and tumor progression in intrahepatic cholangiocarcinoma

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Background and Aims: Cholangiocarcinoma (CCA) comprises a heterogeneous group of malignant tumors with dismal prognosis. During the last years, different studies have highlighted the key role of the immune system in the development of intrahepatic CCA (iCCA) and several combinational therapies targeting the tumor microenvironment (TME) have shown promising results for anti-cancer therapy. In this regard, the macrophage receptor with collagenous structure (MARCO) is a class A scavenger receptor found on particular subsets of macrophages that has been described to play a determining role in macrophage polarization and consequently in adaptive immune responses in many solid tumors. However, its role in iCCA is still unknown. This study aims to unravel the role of MARCO in iCCA development and progression.

Method: The cell-type specific *MARCO* expression was examined in iCCA human tumors by using publicly available single-cell RNA sequencing data from different studies and *MARCO*-expressing tumor-associated macrophages (TAMs) were phenotypically characterized. *MARCO* mRNA expression was analyzed in human control and iCCA liver tissue samples and associated to different immune cell types and immune-functionality scores employing state-of-the-art technologies as ConsensusTME, TIDE and TIP tools. To study the role of MARCO in murine cholangiocarcinogenesis, wild type (WT) and *Marco*$^{-/-}$ mice were subjected to 3 different iCCA murine models and flow cytometry analysis of the TME was carried out to characterize different lymphocytic and myeloid populations.

Results: Single-cell RNA sequencing data indicate that *MARCO* is expressed in a specific subtype of TAMs in patients with iCCA. Besides, high *MARCO* expression levels in the liver samples of patients with iCCA are linked with worse clinical outcome. In line with this, *MARCO* expression in human iCCA tumors is associated to cell types involved in tumor progression such as M2 macrophages, and related with T cell dysfunction. Regarding the potential role of MARCO in murine models of iCCA, *Marco*$^{-/-}$ mice show a trend to be protected from iCCA development, the mechanisms behind this effect being likely associated to a reduction of the innate immune cells such as $CD9^{+}Ly6C^{-}F4/80^{+}$ resident macrophages and type-2 innate lymphoid cells (ILC2). Noteworthy, in a context of a syngeneic orthotopic experimental

model, *Marco*$^{-/-}$ mice exhibit a reduced presence of immune checkpoint molecules in innate and adaptive immune cells, including a lower percentage of PD-L1^{+}Ly6C^{-}F4/80^{+} resident macrophages and, PD-1^{+} and CTLA-4^{+} cytotoxic CD8^{+} T cells in comparison to WT mice.

Conclusion: High *MARCO* expression is associated to a worse outcome in patients with iCCA and is associated to an immunosuppressive TME. Importantly, *Marco*$^{-/-}$ mice display a reduced presence of immunosuppressive cell populations. Therefore, MARCO arises as a novel therapeutic target for iCCA.

P04-08
Incidence rates, trends, and survival for etiology-specific hepatocellular carcinoma and cholangiocarcinoma in the Unites States

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Background and Aims: Liver cancer is a prominent cancer in the US especially among non-White populations with ample variation in patterns by sex, age, and race for etiology-specific hepatocellular carcinoma (HCC). However, cholangiocarcinoma (CC) is on the rise and there is limited data on its epidemiology on a population basis. Florida is the 3rd largest state in the US in population and ranks second in annual number of newly diagnosed ICC cases.

Method: The totality of Florida cancer registry data from 2010–2018, including 14,420 cases of HCC, 2,930 of ICC, and 2,166 of ECC, were linked with population-based discharge statewide data and the department of health viral hepatitis data with 88.2% of cases successfully matched. We analyzed incidence, all-cause 5-year survival (lifetable method) and trends (using joinpoint regression) for HCV-, HBV-, alcohol- and NAFLD-related HCC, as well as total intrahepatic CC and extra-hepatic CC. Because overlap between the different causes of HCC was frequent, a hierarchical classification was used to identify each subject's HCC predominant cause.

Results: HCV cases accounted for most cases of HCC (47%), followed by NAFLD (27%) and alcohol (13%). However, since 2017 NAFLD is the number one cause of HCC in women. HCV-HCC age-adjusted incidence rates were particularly high among US-born males of Black, Puerto Rican, and Mexican ancestry. Overall trends are increasing for NAFLD-HCC (+4.3% annually), alcohol-HCC (+6.0%), and ICC (+7.0%), are stable for ECC and HBV-HCC, and have been decreasing since 2015 for HCV-HCC (-9.6%). Five-year age-adjusted survival remains poor for all types of liver cancer: HCC 18.5% (95%CI 17.7-19.3), Intra-hepatic CC 11.0% (9.2-12.8), and extra-hepatic CC 12.9% (10.5-15.3). For HCC, 5-year survival varied by cause being notably low for the HCV-alcohol combination at 11.4% (9.6-13.1).

Conclusion: Population patterns of liver cancer are considerably heterogeneous; characterization and prevention of liver cancer are limited by its consideration as a single disease. Liver cancer prognosis remains uniformly poor. Priorities should include screening for risk factors and HCC and developing a better understanding of cholangiocarcinoma.

P04-09
AKR1B1 drives hyperglycemia-induced metabolic reprogramming in initiation and progression of NAFLD-associated hepatocellular carcinoma

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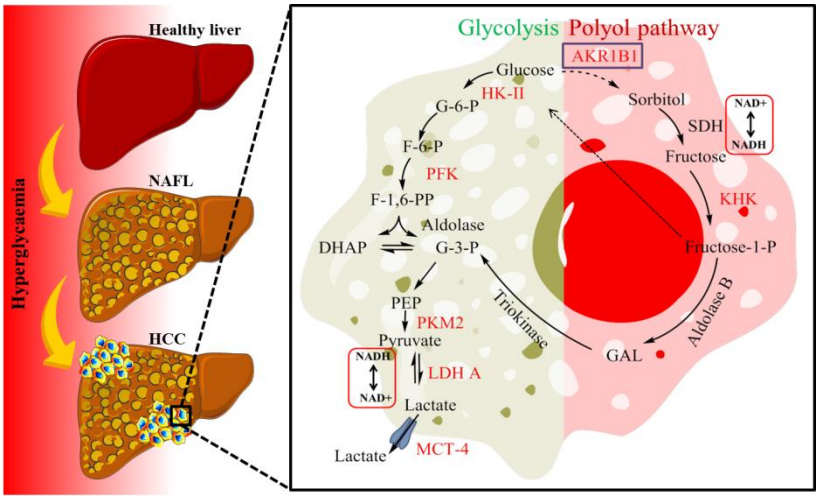
Background and Aims: Emerging epidemiological evidence suggests that the progression of NAFLD/NASH-associated HCC positively correlates with the patient's glycemic index. However, the mechanism behind this progressive pathological alteration is poorly understood. It has shown that the polyol pathway master regulator, AKR1B1 is over-expressed in hyperglycemia and responsible for most of diabetic complications. Hence in the present study, we have investigated the role of AKR1B1 in metabolic switching associated with NAFLD/NASH and in the progression of HCC.

Method: The expression of AKR1B1 in NAFL/NASH, HCC, and HCC with diabetes mellitus patient's liver and plasma were estimated. The role of AKR1B1 in the metabolic switching of HCC cell lines was assessed through media conditioning and lentiviral transfection. Standard inhibitor epalrestat or investigational drug NARI-29 (4-((Z)-5-((Z)-2-Cyano-3-phenylallylidene)-4-oxo-2-thioxothiazolidin-3-yl) benzoic acid) was utilized to elucidate the effect of AKR1B1 inhibition in hepatocarcinogenesis. A proteomic approach was applied for an in-depth investigation of the involved metabolic pathway and to evaluate the therapeutic efficacy of pharmacological inhibitors. Preclinically, a high fructose diet (HFrD) fed in combination with a diethyl nitrosamine (DEN) induced mouse model was developed to investigate the role of AKR1B1 in the hyperglycemia-mediated metabolic switching in the pathobiology of NAFLD and its progression to HCC.

Results: A significant increase in the expression of AKR1B1 was observed in NAFL/NASH, HCC, and HCC-DM tissue samples compared to non-involved adjacent tissues indicating its role in the disease progression. Moreover, a statistically significant elevation of AKR1B1 was observed in NAFLD, NAFLD-associated HCC, and HCC-DM plasma samples compared to normal control. Mechanistically, *Invitro* assays revealed that AKR1B1 modulates the Warburg effect, mitochondrial dynamics, TCA cycle, and lipogenesis to promote hyperglycemia-mediated fatty liver and cancer progression. A pathologically increased expression of AKR1B1 was observed in experimental NAFL-HCC, and expression was positively correlated with high blood glucose levels. HFrD+DEN-treated animals also exhibited statistically significant elevation of metabolic markers and carcinogenesis markers. However, AKR1B1 inhibition with EPS or NARI-29 has inhibited cellular metabolism in vitro and in vivo models.

Conclusion: Pathological AKR1B1 modulates hepatic glucose metabolism to promote NAFLD-associated hepato-carcinogenesis. Aldose reductase inhibition modulates glucose metabolism to prevent the pre-cancerous hepatocyte formation. Hence EPS and NARI-29 could be promising AKR1B1 inhibitors for controlling aberrant metabolism and treating NAFLD-associated HCC.

Figure:


Healthy liver
NAFL
HCC
Hyperglycaemia
Glycolysis
Polyol pathway
AKR1B1
Glucose
HK-II
G-6-P
F-6-P
PFK
F-1,6-PP
Aldolase
DHAP
G-3-P
PEP
PKM2
Pyruvate
NADH
NAD+
LDH A
Lactate
Lactate
MCT-4
Sorbitol
SDH
NAD+
NADH
Fructose
KHK
Fructose-1-P
Aldolase B
GAL
Triokinase

P04-12
The potential of combined treatments between Src tyrosine kinase inhibitors and sorafenib in heterogeneous liver cancer cells

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Background and Aims: Sorafenib (SOR) is the most common first-line therapy for advanced hepatocellular carcinoma (HCC). However, efficacy of this multikinase inhibitor remains modest. Furthermore, a substantial number of HCC patients does not benefit due to drug resistance, attributed to HCC cellular complexity and heterogeneity. Therefore, new therapies or combination therapies to SOR to improve survival of advanced HCC patients are still warranted. This study aims to explore combination effects Src tyrosine kinase inhibitors and SOR in overcoming cellular heterogeneity of HCC.

Method: *In silico* analysis of published datasets on HCC classifications using protein-protein interaction (PPI) network analysis identified members of the Src family of tyrosine kinases (SFKs) as potential targets of therapy. The relevance of these targets was assessed *in vitro* in six hepatic cell lines, representing HCC heterogeneity: S1/TGFβ-Wnt subtype (HLE, HLF and JHH6), S2/progenitor subtype (HepG2 and Huh7) and an immortalized hepatocyte (IHH) as control. Gene expression analysis was conducted to assess the baseline expressions of the SFKs in HCC cells vs hepatocyte. All cell lines were treated with Src inhibitors, saracatinib (SAR) and dasatinib (DAS), with concentration ranging from 0.02 to 10.00 µM. To define the efficacy of combination therapy of Src inhibitors and SOR, co-treatments were evaluated. Lethal concentration (LC_{50}) was determined considering the effects of the drug as single or combination treatment.

Results: *In silico* PPI analysis proposed four members of SFKs: ASV1, YES, FYN and FGR, as potential molecular targets. Gene expression analysis revealed that in HCC cells, ASV1 and YES was 10 and 3-fold higher ($p<0.05$) as compared to IHH, respectively, while FYN and FGR were downregulated (1.5 and 2-fold less, respectively). Following *in vitro* treatments, both SAR and DAS treatment alone were not significantly toxic to the cells. Similar effect was noticed for 10 µM SOR treatment alone, showing a modest cell viability reduction between 70 and 88% in HepG2, Huh7, HLE and IHH cells while it was not changed for HLF and JHH6. Interestingly, combination therapy between these Src inhibitors with SOR resulted to a dose dependent response to the drugs. HCC cell lines HepG2 and HLE were the most sensitive to co-treatments, with LC_{50} values for HepG2 and HLE of 0.06 and 0.6 µM for SAR-SOR and 0.01 and 0.02 µM for DAS-SOR, respectively. For HLF, JHH6 and Huh7 the LC_{50} values were 5.7, 4.5 and 2.6 µM respectively for SAR-SOR, while for DAS-SOR, their LC_{50} values were 1.8, 4.6, and 1.2 µM, respectively. It is important to notice that in IHH control cells, the SAR-SOR combination was less toxic compared to DAS-SOR treatment, with LC_{50} of 4.8 vs 0.1 µM, respectively.

Conclusion: Src tyrosine kinase inhibitors combined with SOR therapy may provide a potential treatment advantage in HCC to comprise cellular heterogeneity.

P04-13
Investigation of novel hepatoblastoma chemosensitizers based on the inhibition of ABC pumps-mediated drug efflux

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Background and Aims: The poor prognosis of about one-third of patients with hepatoblastoma (HB) is mainly due to the refractoriness of this cancer to neoadjuvant chemotherapy, which is commonly based on cisplatin and doxorubicin. In previous studies, we have demonstrated that the high expression of drug export pumps belonging to the ABC superfamily of proteins, mainly MDR1, MRP1, and MRP2, plays a primary role in HB chemoresistance. The aim of this study was to search for non-toxic inhibitors of these transporters and to evaluate in vitro their ability to sensitize HB cells to antitumor chemotherapy.

Method: Cell lines with endogenous or chemically induced high expression of MDR1 (HepG2/DR) or MRP1/MRP2 (HB-282) were used. Gene expression was determined by RT-qPCR, Western blot, and immunofluorescence. ABC-mediated transport activity was determined by flow cytometry using fluorescent substrates and specific inhibitors. Known inhibitors of ABC pumps were used as controls. In silico analysis by molecular docking was performed to look for potential ABC inhibitors using homology models for these proteins and a library of 40,000 natural or semi-synthetic compounds. Potentially harmful compounds were discarded based on toxicity prediction using the online tool ProTox-II. Cell viability was determined by the MTT-formazan and sulforhodamine B assays. SynergyFinder 3.0 was used to assess drug combination synergy.

Results: Besides known MDR1 inhibitors (verapamil, elacridar, and tariquidar), 11 novel compounds, among 40 potential inhibitors studied, significantly reduced rhodamine-123 efflux from HepG2/DR cells. Among these with no cytotoxic effect, only CCL-40 was able to slightly increase the sensitivity of cells to doxorubicin. However, CCL-17 and CCL-24, both tyrosine kinase inhibitors, markedly enhanced the cytostatic effect of doxorubicin, which was due to a synergistic mechanism. Regarding MRP1 and MRP2, the molecular docking study identified 1,000 compounds with potential interaction with these pumps. Among them, the best eight compounds, based on low binding energy to both proteins, low predicted toxicity, and commercial availability, were further studied. The results revealed that two of them, CCL-45 and CCL-46, significantly reduced MRP1/MRP2-mediated calcein efflux in HB-282 cells. The chemosensitizing potency of CCL-45 was even stronger than the typical MRP1/MRP2 inhibitor MK-571.

Conclusion: Inhibition of ABC drug export pumps, such as MDR1, MRP1, and MRP2, by several non-toxic natural compounds and drugs commonly used in the clinic for other purposes, could be a helpful strategy to overcome the lack of response to chemotherapy in HB patients.

P04-14
Inhibition of lysophosphatidic acid receptor 6 upregulated by the choline-deficient L-amino acid-defined diet prevents hepatocarcinogenesis in mice

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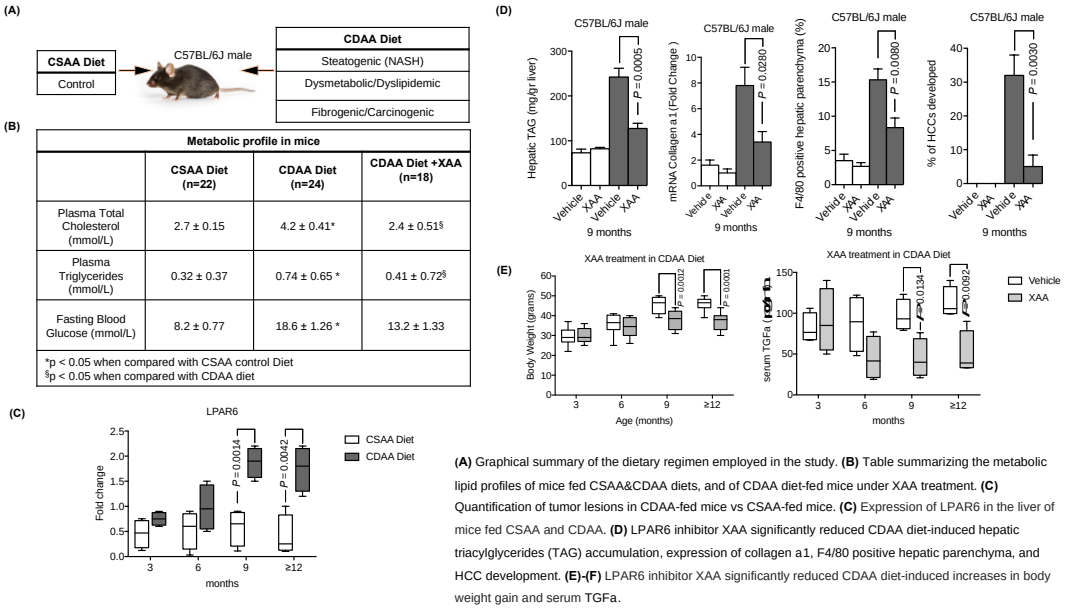
Background and Aims: Hepatocellular carcinoma (HCC) is one of the most disseminated neoplasms worldwide, with alarming epidemiological trends. Traditional pharmacological approaches show scarce effectiveness and many side effects, therefore new effective molecules with fewer side effects are needed to support traditional methods. We previously demonstrated that lysophosphatidic acid (LPA) promotes hepatocarcinogenesis by binding to LPA receptor 6 (LPAR6). We also reported that 9-xanthylacetic acid (XAA), an antagonist of LPAR6, inhibits the growth of HCC. Here, we describe the role of LPAR6 in modulating the effects of steatogenic diet and the effects of XAA in mice.

Method: C57BL/6J male mice were fed a choline-sufficient amino acid-restricted diet (CSAA diet, n=22) and a choline-deficient amino acid-restricted diet (CDAA diet, n=24). CDAA diet is known to cause hepatic steatosis, metabolic abnormalities, fibrosis, and HCC development. Mice on both diets were treated with XAA at a dose of 5 mg/kg body weight.

Results: Our data suggest that CDAA diet-induced metabolic imbalance stimulates LPAR6 expression in mice, whereas XAA counteracts hepatic lipid accumulation, fibrosis, inflammation, and HCC development. We provide evidence linking diet-induced metabolic stress to increased LPAR6 expression, and recognize for the first time LPAR6 as a relay of metabolic distress, the “missing link” between diet and HCC.

Conclusion: Our results suggest that diet-induced metabolic distress stimulates hepatic LPAR6 expression, which is associated with the acquisition of a NASH/NAFLD phenotype in mice, ultimately leading to the development of HCC. In conclusion, we identify a role for LPAR6 in hepatocarcinogenesis and suggest a new prevention option for HCC through pharmacological LPAR6 inhibition in combination with lifestyle regimens.

Figure:



(A) Graphical summary of the dietary regimen employed in the study. **(B)** Table summarizing the metabolic lipid profiles of mice fed CSAA&CDAA diets, and of CDAA diet-fed mice under XAA treatment. **(C)** Quantification of tumor lesions in CDAA-fed mice vs CSAA-fed mice. **(C)** Expression of LPAR6 in the liver of mice fed CSAA and CDAA. **(D)** LPAR6 inhibitor XAA significantly reduced CDAA diet-induced hepatic triacylglycerides (TAG) accumulation, expression of collagen a1, F4/80 positive hepatic parenchyma, and HCC development. **(E)-(F)** LPAR6 inhibitor XAA significantly reduced CDAA diet-induced increases in body weight gain and serum TGFa.

P04-15
Curcumin; an epigenetic regulator of lncRNA HOTAIR expression in HCC through LSD1/H3K9me2 axis

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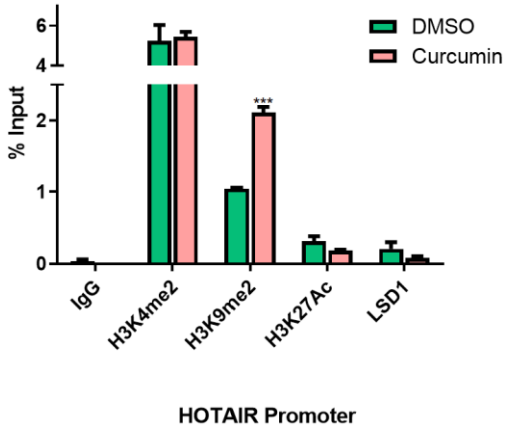
Background and Aims: Curcumin, a polyphenolic derivative produced from Curcuma longa, is reported to have an epigenetic modulatory role in multiple cancers. For instance, curcumin exerts its anti-cancer activity through regulating DNA methyltransferases (DNMT) as well as post-translational histone modifications. Lysine specific demethylase-1 (LSD1) activity is based on a context-dependent co-regulatory function in transcription, acting as a co-activator or co-repressor. LSD1 can specifically demethylate mono-or di-methyl H3K4, resulting in repression of gene expression, and H3K9 histone marks, inducing the expression of the downstream regulated genes. Hox Transcript Antisense Intergenic RNA (HOTAIR) is a long non-coding RNA that is widely expressed in several tumors and postulated to be involved in tumor pathogenesis. This study aims to investigate the epigenetic mechanism by which curcumin regulates the expression of HOTAIR in hepatocellular carcinoma (HCC) through LSD1 as an intermediate player.

Method: MTT assay was used to assess the curcumin concentration required for 50 % cell viability in Huh-7 cells. RT-qPCR and western blotting were used to detect gene and protein expression upon curcumin treatment for Huh-7 cells. ChIP-immunoprecipitation followed by ChIP-qPCR were used to verify the epigenetic regulatory role of curcumin in HOTAIR expression using specific antibodies targeting LSD1, H3K9me2, H3K4me2, H3K27Ac and IgG as a negative control.

Results: 30 µM curcumin treatment in Huh-7 cells was able to significantly repress the expression of both HOTAIR and LSD1 ($p = 0.0005$ and 0.0001 respectively). Western blotting showed downregulation of LSD1 in Huh-7 cell lines upon curcumin treatment. However, curcumin treatment was not able to induce a significant change in the universal levels of both histone marks H3K9me2 and H3K4me2. Furthermore, inhibition of LSD1 activity by C12 in Huh-7 cells was able to downregulate the expression of HOTAIR ($p = 0.003$). Following curcumin treatment in Huh-7 cells, ChIP qPCR has revealed a significant enrichment in H3K9me2 histone mark at HOTAIR promotor ($p = 0.0002$). Nonetheless, no significant change in H3K4me2 histone mark enrichment at HOTAIR promotor. Moreover, curcumin treatment showed a tendency to halt the enrichment of H3K27Ac and LSD1 at HOTAIR promotor.

Conclusion: Our study revealed a potential epigenetic modulatory role for curcumin in HOTAIR expression in HCC. The regulatory activity of curcumin could be manifested by LSD1 as an intermediate player and a subsequent enrichment of H3K9me2 histone mark at HOTAIR promotor, and hence, downregulated expression of oncogenic lncRNA HOTAIR. This study showed that curcumin can be regarded as a potential adjuvant phytochemical therapy in HCC.

Figure:


DMSO
Curcumin
% Input
6
4
2
1
0
***
IgG
H3K4me2
H3K9me2
H3K27Ac
LSD1
HOTAIR Promoter

P05-03
Interplay between genetic and immune factors in the tumor microenvironment and their prognostic value in hepatocellular carcinoma

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Background and Aims: Hepatocellular carcinoma (HCC) is a fatal disease characterized by early genetic alterations in telomerase reverse transcriptase promoter (TERTp) and β-catenin (CTNNB1) genes and by changes in immune cell landscape in the tumor microenvironment. As a novel approach, we wanted to assess patient survival influenced by combined presence of mutations and densities of CD3+ T-lymphocytes and CD20 B-lymphocytes.

Method: Tissue samples were obtained from 67 HCC patients who had undergone resection and no neo-adjuvant treatment prior operation. We analysed TERTp mutations, TERTp rs2853669 polymorphism, and CTNNB1 mutations. After immunohistochemical staining, the densities of CD3+ T-lymphocytes and CD20 B-lymphocytes were estimated stereologically in the tumor center (TC) and invasive margin (IM). These variables were evaluated as predictors for time to recurrence (TTR), disease free survival (DFS) and overall survival (OS).

Results: TERTp mutations were found in 75.8 % and CTNNB1 mutations in 35.6 % of the patients. All individual factors showed their own predictive scores but combined genetic and immune factors further improved survival, showing higher predictive values. Combination of CTNNB1 mutations and high CD3+ cells densities showed high predictive values for TTR and DFS in comparison to patient without mutation and with low densities of CD3+ T cells. On the other hand, high CD20+ cells densities combined with CTNNB1 mutations were associated only with TTR. Presence of GG genotype in TERTp rs2853669 polymorphism and high CD3+ cells densities in TC was associated with longer DFS and OS. Additionally GG genotype in TERTp rs2853669 polymorphism combined with high CD20 cells densities in TC showed longer TTR and longer DFS in comparison to AA genotype and low CD20 B-lymphocytes densities.

Conclusion: The results show that factors both in the tumor and in its microenvironment may influence patient outcome in HCC. The best predictive values for TTR were obtained by combining data for rs2853669 and CD3+ cell densities in TC. Moreover, best predictive score for DFS and OS were obtained by combining data for rs2853669 with CD3+ T-lymphocytes densities in TC. We were able to analyse the most common somatic mutations and two vital adaptive immunity cell markers in this first analysis calling for further validation of the integrative approach. In the future, many more cell types of the tumor microenvironment need to be tested in combination with the genetic markers.

Figure:

Variable	Group	N	TTR HR	DFS HR	OS HR
CTNNB1 Mutation + CD3+ (TC)	CTNNB1(-)/CD3TC(LD)	8	1.0	1.0	1.0
	CTNNB1(+)/CD3TC(HD)	18	**0.22 (p=0.02)**	**0.35 (p=0.04)**	1.67 (p=0.5)
CTNNB1 Mutation + CD3+ (IM)	CTNNB1(-)/CD3IM(LD)	11	1.0	1.0	1.0
	CTNNB1(+)/CD3IM(HD)	17	0.33 (p=0.1)	0.57 (p=0.2)	1.68 (p=0.4)
CTNNB1 Mutation + CD20+ (TC)	CTNNB1(-)/CD20TC(LD)	10	1.0	1.0	1.0
	CTNNB1(+)/CD20TC(HD	17	**0.23 (p=0.03)**	0.49 (p=0.2)	1.41 (p=0.6)
CTNNB1 Mutation + CD20+ (IM)	CTNNB1(-)/CD20IM(LD)	10	1.0	1.0	1.0
	CTNNB1(+)/CD20IM(HD)	16	**0.23 (p=0.05)**	0.49 (p=0.2)	1.40 (p=0.6)
rs2853669 + CD3+ (TC)	AA/CD3TC(LD)	3	1.0	1.0	1.0
	GG/CD3TC(HD)	11	0.13 (p=0.1)	**0.08 (p=0.001)**	**0.06 (p=0.003)**
rs2853669 + CD20+ (TC)	AA/CD20TC(LD)	8	1.0	1.0	1.0
	GG/CD20TC(HD)	13	**0.12 (p=0.02)**	**0.25 (p=0.01)**	0.50 (p=0.2)

P05-05
Association of TP53 with defective long chain 3-hydroxy acyl-CoA dehydrogenase induced non-cirrhotic hepatocellular carcinoma

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Background and Aims: Little is known about non-alcoholic fatty liver disease (NAFLD) without cirrhosis as a risk for development of hepatocellular carcinoma (HCC). The incomplete understanding of underlying mechanisms and associated molecular events that may lead to HCC associated with hepatic steatosis are hampering the surveillance and management of HCC in NAFLD patients and becoming a major public health concern. Defects in mitochondrial fatty acid oxidation leads to development of hepatic steatosis. Studies were planned to understand molecular events involved in non-cirrhotic HCC.

Methods: Male heterozygous (HT) long chain 3-hydoxy acyl-CoA dehydrogenase (LCHAD) (n=10) and wild-type (n=10) mice were maintained on chow diet. Liver steatosis was assessed by H&E and oil red O staining. Livers were also inspected for macroscopic lesions and assessed by histological examination. Wild-type and cancer free HT mice livers were analysed by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and proteins with significant changes in abundance were identified by mass spectrometry. Differentially expressed proteins between wild-type and non-cancer HT mice were subjected to Ingenuity Pathway analysis (IPA) software (version 01-14). cDNA array study of HCC patients was conducted to assess expression of HADHA transcripts.

Results: None of the wild-type mice developed steatosis and HCC, whereas livers from all HT mice showed moderate steatosis starting at 3 months progressing to severe steatosis at older age, but none had necro-inflammation or fibrosis. Further, 20% HT mice developed liver masses with histological features of HCC at 14-18 months. Abundance of twenty-four proteins was significantly different ($p<0.01$) between wild-type and HT mice. Proteins of glycolysis, stress pathways and tumorigenesis were up-regulated, whereas proteins involved in mitochondrial respiration, fatty acid oxidation, oxidative stress protection, and tumour suppression were down-regulated. IPA analysis identified hepatotoxicity and changes in the Signalling Network1 involved with metabolic diseases and organismal injury as well as Signalling Network2 involved with cancer, haematological diseases, and organismal injury/abnormalities. Only one cross interacting gene *TP53* was identified by merging both networks. Hepatotoxicity report identified enrichment of four functional groups, which are changes in redox biology, steatosis, hyperplasia/hyperproliferation, and hepatocellular carcinoma. cDNA array study demonstrated significant reduction of HADHA transcripts in HCC patients.

Conclusion: The study suggests that impaired fatty oxidation may play a role in development of HCC associated with steatosis without cirrhosis.

P05-06-YI
Preferential effects of PARP-1 inhibition in KRAS-mutated intrahepatic cholangiocarcinoma is mediated by CHK1 kinase

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Background and Aims: Intrahepatic cholangiocarcinoma (iCCA) is the second most common primary liver cancer with an increasing incidence over recent years. Due to the complexity of iCCA pathogenesis and the pronounced genetic heterogeneity treatment options are still limited. Activating KRAS mutations are among the most abundant genetic alterations in iCCA and are associated with early recurrence, poor response to chemotherapy, and reduced overall survival, highlighting the need for novel therapeutic approaches. Poly(ADP-ribose)polymerase-1 (PARP-1) is frequently observed to be upregulated in iCCA. Evidence indicate potential therapeutic relevance for PARP-1 inhibition in iCCA that preferentially affects KRAS-mutated cancers, but exact mechanisms remain unknown.

Method: PARP-1 depletion was generated by siRNA and CRISPR/Cas9-mediated knockdown/knockout in KRAS-mutated and non-mutated iCCA cell lines. Functional assessment of PARP-1 knockout and inhibition of tumorigenic potential was analyzed by viability assay and colony and sphere formation. RNA sequencing was employed to further decipher PARP-1 regulation. To investigate the impact of PARP-1 deficiency in KRAS-driven tumorigenesis, PARP-1 knockout mice were combined with an inducible KRAS-driven mouse model using hydrodynamic tail vein injection. Molecular analyses including transcriptome profiling were employed to further investigate molecular mechanisms.

Results: Significant upregulation of PARP-1, as well as enrichment of genes related to PARP-1 activation, was observed in iCCA tissue and KRAS-mutated cell lines. Knockout of PARP-1 in KRAS-mutated cells led to a reduction in colony and sphere formation. Moreover, KRAS-mutated cell lines showed higher sensitivity to PARP-1 inhibition. In vivo PARP-1 deficiency considerably impaired biliary carcinogenesis and induced a shift from dominant iCCA towards HCC phenotype in a KRAS-dependent manner. Transcriptome analyses of CRISPR/Cas9 PARP-1 knockout clones and in vivo tumors revealed differential expression of DNA damage response pathways (e.g. CHK1) as well as cellular pathways affected by PARP-1, (inflammation, oxidative stress, cell death signaling). The most prominent candidate regulating PARP1 in KRAS cell lines and tumors appeared to be CHK1 kinase, further validated by qRT-PCR, western blot, and drug-screening assays.

Conclusion: Together, these findings suggest an unrecognized prognostic and therapeutic role of PARP-1 in iCCA patients with oncogenic KRAS signaling and unveil the potential mechanism of PARP-1 regulation by CHK1 kinase.

P05-07-YI
Developing image-guided precision radiotherapy in preclinical liver cancer models

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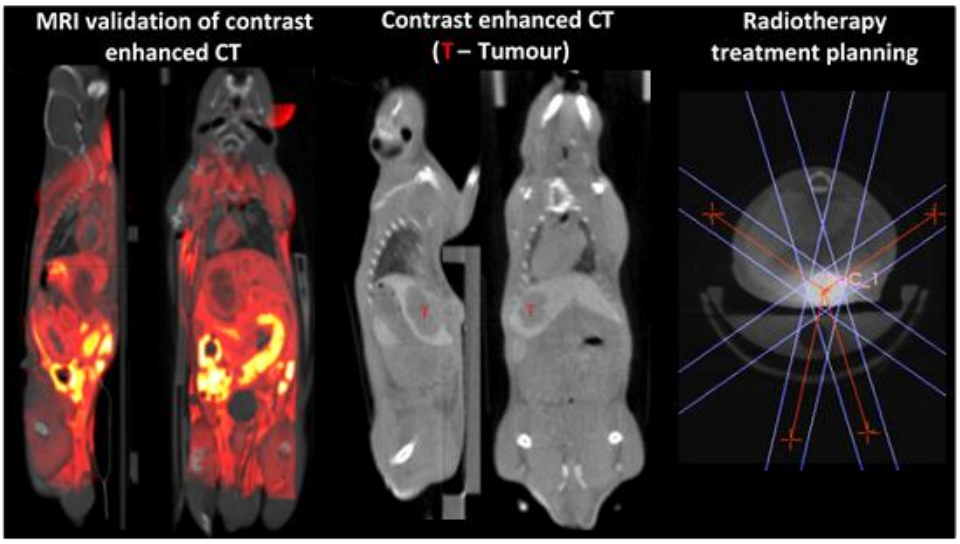
Background and Aims: Optimal treatment for Hepatocellular carcinoma (HCC) is curative, either resection, liver transplantation or local ablation, however, only ~20% of patients are eligible for this. The majority of patients present with incurable disease, for whom transarterial chemoembolisation or systemic chemotherapy are palliative options. Unfortunately, both have low responses and high rates of recurrence and progression. Stereotactic Body Radiation Therapy (SBRT) is an emerging treatment option for a subset of HCC patients who are not eligible for resection or other local treatments. While prospective trials are required to better understand the role of SBRT in HCC, retrospective studies report that this treatment modality has both clinical efficacy and feasibility. SBRT can provide good tumour control, but with recurrent and disseminated disease, there is a significant opportunity for integrating SBRT into multimodal combination therapy. With this, there is a significant research opportunity in the preclinical space for models of precision radiotherapy in HCC. Our aim is to develop a clinically relevant model to study targeted radiotherapy and systemic therapies in the treatment of HCC.

Method: We have optimised an orthotopic transplant model injecting a mouse-derived HCC cell line into the immunocompetent murine liver. Using co-registered MRI/CT scans we have validated imaging-contrast agents to determine their suitability for CT-guided tumour identification. A timepoint optimization study examining toxicity to the healthy liver, tumour and organs-at-risk was performed on a small animal radiation research platform delivering 20Gy single fraction irradiation. Radiation-induced damage was assessed using immunohistochemistry and liver biochemistry.

Results: We have established a syngeneic orthotopic transplant model whereby transplantation of a murine-derived HCC cell line into the murine liver gives rise to an anatomically accurate liver tumour in an immunocompetent setting. In keeping with clinical management and treatment planning, we have successfully implemented intravenous contrast enhanced imaging in our model to reliably detect and delineate liver tumours, enabling long-term longitudinal CT imaging follow-up. We have shown that 20Gy single fraction irradiation (arc beam) induces significant DNA-damage in tumours compared to non-irradiated tumour controls, with minimal off-target dosing. Finally, we report, irradiation associated infiltration of CD8+ cells into the tumour 2 weeks after radiotherapy.

Conclusion: Our orthotopic transplant model is an exemplar platform to study targeted radiotherapy of HCC and its combination with systemic therapies including immunotherapy.

Figure:


MRI validation of contrast enhanced CT
Contrast enhanced CT (T – Tumour)
Radiotherapy treatment planning

P05-08
Cytokine levels and circulating DNA profiling in plasma as biomarkers of response to immunotherapy in hepatocellular carcinoma

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Background and Aims: The combinations of anti-PD-L1 antibody (atezolizumab) and VEGF-neutralizing antibody (bevacizumab) and anti-PD-L1 antibody (durvalumab) and anti-CTLA-4 (tremelimumab) have become first line options for advanced hepatocellular carcinoma (HCC). Our aim was to identify potential serological markers of response to immune checkpoint inhibitors (ICI).

Method: Prospective cohort of 25 patients treated with ICIs (Nivolumab (n=14), Atezolizumab/Bevacizumab (n=8), Durvalumab/Tremelimumab (n=2) and Lenvatinib/Pembrolizumab (n=1)). Plasma samples were collected at the beginning and after 3 months of ICI treatment. 24 inflammatory cytokine levels were analyzed by ELISA, the levels of circulating cell free DNA (cfDNA), circulating tumor DNA (ctDNA) and percentage of TERT mutation by ddPCR. Basal cfDNA profiling from 21 of these patients was analyzed by Onco-500 TruSight.

Results: 84% of patients were male, median age was 71 years and 76% were BCLC-C at the beginning of ICIs treatment. HCV infection was the most frequent etiology (52%). Median follow-up was 17 months. 8% presented complete radiological response (CR), 20% partial radiological response (PR), 44% stable disease (SD) and 28% radiological progression (PD) as best radiological outcome (RECIST 1.1). Baseline CTLA-4 levels were significantly higher in patients presenting radiological progression [mean (SD)] [76.55 (150.84) in PD and 0.15 (0.67) pg/ml in no PD](p<0.05). 3 months post-treatment, MCP-1 levels were significantly lower in patients presenting PD [49.25 (25.73)] than in patients presenting CR/PR/SD [73.73 (86.34) pg/ml] (p<0.05) and TNF-α levels were significantly higher in those patients [110.4 (239.7) vs 49.9 (189.43) pg/ml] (p<0.05). Baseline cfDNA levels were significantly different between patients presenting radiological response [2.3(0.58) ng/ul] vs patients SD/PD [8.95(6.89) ng/ul]. Higher levels of cfDNA than 3.04ng/ul were associated with a poorer overall survival (p<0.005). These differences were also present after 3 months under therapy. Regarding cfDNA sequencing patients with CR/PR had significantly more copy number variation (CNV) than those without it (97 vs 1) (p<0.05). Mutations in CTNNB1 were present in 100% of patients showing PD, but only in 53% of those presenting with SD/PR/CR, and patients presenting PD had significantly more pathological mutations in CDKN2A (67% vs 7%) (p<0.05).

Conclusion: Basal levels of cfDNA, CTLA-4, CDKN2A mutations and CNV are significantly different between patients with and without radiological response to ICIs treatment. Levels of MCP-1, TNF-α and total amount of cfDNA and ctDNA after three months of ICIs treatment are significantly different in patients presenting radiological response. Analysis of cfDNA and cytokines could help to identify HCC patients benefiting more of immunotherapies. Deeper molecular analysis are ongoing.

P05-10
Correction for length bias reduces the mortality benefit from Hepatocellular Carcinoma surveillance

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Background and Aims: Hepatocellular carcinoma (HCC) surveillance in patients with various forms of chronic liver disease and cirrhosis is recommended by international guidelines. A recent meta-analysis has shown a significant survival benefit from surveillance. However, the results do not adjust for length time bias. We aim to assess the effect of length bias on estimates of survival benefit using new estimates of the proportion of slow growing HCCs.

Method: We analysed papers which had been used in a previous meta-analysis and extracted survival data from papers which filled the criteria for our model. Included cohorts needed to report set time mortality of both surveillance and non-surveillance groups. We used a validated statistical model to estimate the effect of length bias on measures of relative mortality risk. In the model we used two published tumour characteristics to estimate an adjusted mortality risk ratio: 1) relative risk of mortality of slow to fast growing tumours (0.7-0.9), and 2) the proportion of slower growing tumours in the study population (0.376 (95%CI 0.345-0.403)).

Results: We found a reduction in the survival benefit associated with HCC surveillance after adjustment for length bias (Figure 1). The mean percentage increase in relative risk estimates for one year, three years, and five years were 3.48 (SD 1.56), 8.02 (SD 12.13), and 10.15 (SD 9.74) respectively. There was significant variability of the effect of length bias between cohorts, largely due to differences in relative sizes of surveillance and control groups. For cohorts with longer follow up times the effect of length bias was found to be greater, with higher estimates in the sensitivity analysis passing 1. Survival benefit decreased with increased follow up time in all cohorts.

Conclusion: We have shown that adjustment for length time bias reduces the benefit of HCC surveillance on patient survival. However, in most of our included studies the survival benefit persisted. Length time bias and the development of methods to adjust for it need greater attention in the absence of substantive randomised controlled trials proving survival benefit. This is of particular importance in the context of the MAFLD epidemic, with early data suggesting that HCCs from this aetiology are slower growing and have a lower mortality than other chronic liver disease aetiologies, increasing the effect of length bias when evaluating survival benefit associated with surveillance.

Figure:

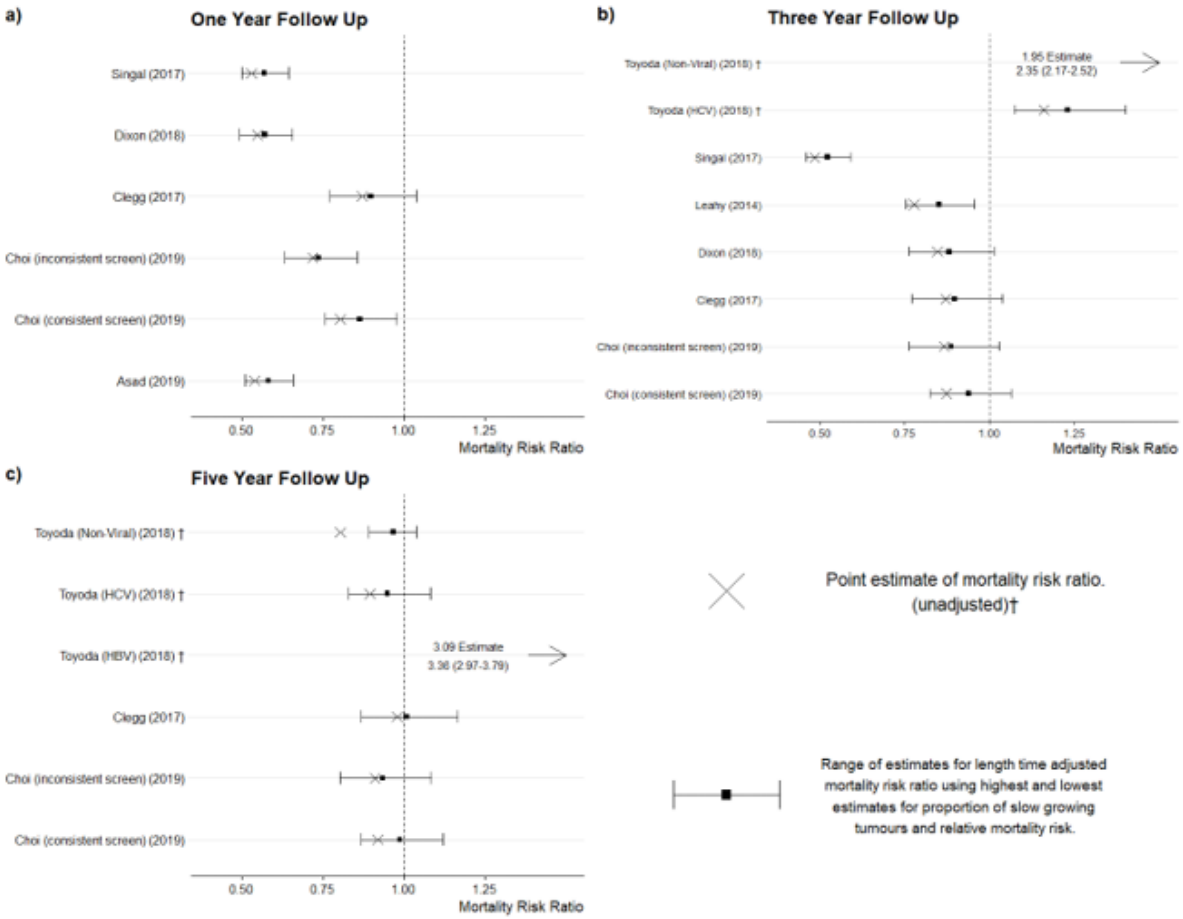

Figure 1: Sensitivity analysis for length time corrected mortality risk ratios, using estimates for proportion of slower growing tumours and relative mortality probability between slow and fast growing tumours. Compared to a point estimate of mortality risk ratio associated with surveillance. †Toyoda et al adjusted set time mortality for lead time bias using novel methodology and did not report unadjusted set time mortality risks.

P05-13
Inhibiting methionine aminopeptidase 2 suppressed VEGFA-mediated angiogenesis by enhancing phosphorylation of eIF2α

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Background and Aims: Our previous experiments demonstrated that in a diethylnitrosamine (DEN)-induced rat cirrhosis-hepatocellular carcinoma (HCC) model, inhibition of methionine aminopeptidase 2 (MetAP2) decreased HCC development by inhibiting cirrhosis progression. MetAP2 inhibits the phosphorylation of eukaryotic initiation factor 2 (p-eIF2α) to enhance the translation, synthesis and maturation of liver proteins. In this study, we hypothesized MetAP2 inhibition could suppress HCC development in cirrhotic tissue by inhibition of VEGFA-mediated angiogenesis.

Method: MetAP2 expression was inhibited by ZGN1345, knocked out by crisper/Cas9 and rescued by MetAP2 expressing plasmid. VEGFA luciferase reporter activity was measured to reflect the translation of VEGFA. Angiogenesis was analyzed with HUVECs. DEN-induced rats received MetAP2 inhibitors ZGN1345 (3 mg/kg) or ZGN-1136 (0.3 mg/kg) and their corresponding controls for 10 weeks. Molecular and histological assessments, and RNASeq were performed. *Ex vivo* Precision-Cut Liver Slices (PCLS) from thioacetamide-induced rat cirrhosis and patient cirrhotic livers were assessed after MetAP2 inhibition.

Results: MetAP2 expression increased with enhanced angiogenesis in cirrhotic livers and HCC nodules in both human patients and the DEN-induced rat model, compared with corresponding controls. After MetAP2 inhibition with ZGN1345 or knockout of MetAP2, p-eIF2α was enhanced in HCC cell line Huh7. Subsequently, an increase in p-eIF2α inhibited the translation of angiogenesis marker VEGFA, which was evidenced by decreased VEGFA luciferase reporter activity after the knockout of MetAP2 in Huh7 cells. After the rescue of MetAP2, the translation of VEGFA was reversed in MetAP2-depleted Huh7 cells, which was attenuated again after MetAP2 inhibition with ZGN1345. As a consequence, MetAP2 inhibition hindered the angiogenesis progress, including inhibiting cell proliferation and migration, endothelial tube formation and spheroid capillary sprouting capacity of HUVECs. Consistently, MetAP2 inhibition increased p-eIF2α in both DEN-induced rat cirrhotic livers and HCC tissues. Fewer vessels were observed, VEGFA expression and angiogenesis was inhibited after MetAP2 inhibition with ZGN1345 in cirrhotic livers and HCC tissues. Assessment of global transcriptomics demonstrated that MetAP2 inhibition down-regulated angiogenesis signatures. Moreover, expression of *Vegfa* decreased in *ex vivo* PCLS from rat and human cirrhotic livers. MetAP2 inhibition with ZGN1136 closely matched and further supported these observations.

Conclusion: MetAP2 inhibition hindered VEGFA-mediated angiogenesis via enhancing phosphorylation of eIF2α, to prevent the progression of cirrhosis to HCC. Our data further suggest that inhibition of MetAP2 has potential as a new prevention target to inhibit the progression of cirrhosis to HCC.

P06-03-YI
Immunotherapy resistance in NASH-HCC is driven by the dysfunctional liver microenvironment of NASH

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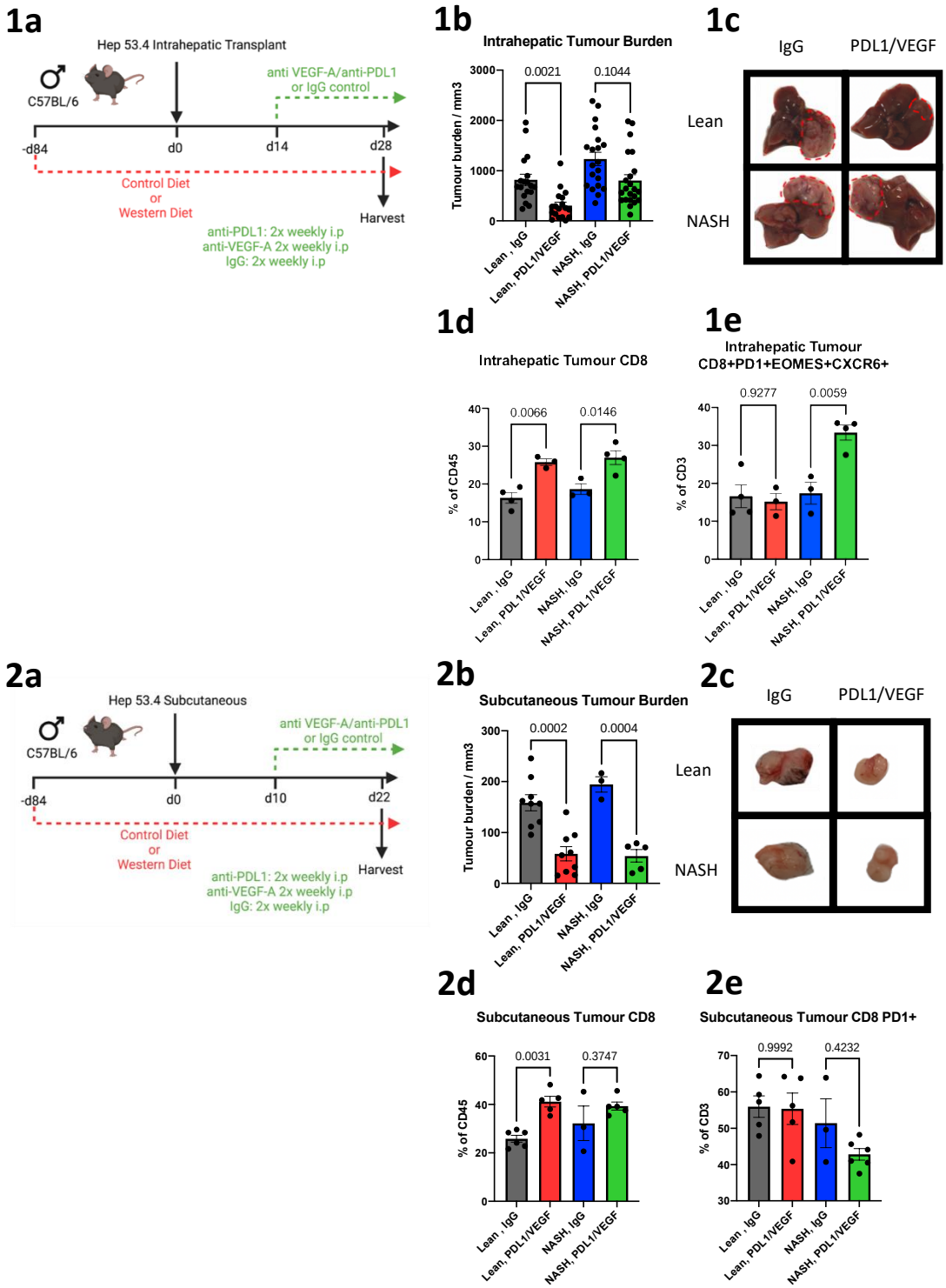
Background and Aims: There is growing evidence suggesting that anti-programmed death ligand 1 (PDL1)/ anti-vascular endothelial growth factor (VEGF) combination therapy in hepatocellular carcinoma (HCC) with underlying non-alcoholic steatohepatitis (NASH) is less effective compared to other aetiologies. Previous work suggests that a subgroup of exhausted CD8+PD1+EOMES+CXCR6+ T cells is responsible in the context of anti-PD1 monotherapy. We aim to explore this further in the context of anti-PDL1/VEGF combination therapy.

Method: C57BL/6J mice were fed either a western diet to induce NASH or fed control diet (lean mice) followed by either intrahepatic or subcutaneous implantation of tumours (Hep 53.4 line). After 2 weeks of tumour growth mice were treated with either anti-PDL1/VEGF or IgG isotype control. Tumour burden was measured and immune characterisation conducted using flow cytometry.

Results: NASH mice with intrahepatic tumours had a poor response to anti-PDL1/VEGF therapy compared to lean mice (figure 1a-c). Analysis showed an influx of CD8 T cells into anti-PDL1/VEGF treated intrahepatic tumours but with a preferential recruitment of CD8+PD1+EOMES+CXCR6+ T cells in NASH intrahepatic tumours (figure 1d-e). On the other hand, both NASH and lean mice with subcutaneous tumours had a good response to anti-PDL1/VEGF therapy (figure 2a-c). Analysis demonstrated an influx of CD8 T cells into the subcutaneous tumours of anti-PDL1/VEGF treated mice. Unlike in the intrahepatic tumours, the proportion of recruited CD8+PD1+ cells were similar in NASH and lean mice (figure 2 d-e).

Conclusion: In our model of NASH-HCC intrahepatic tumours have a poor response to anti-PDL1/VEGF therapy whereas subcutaneous tumours have a good response. This was likely due to a higher proportion of exhausted CD8+PD1+EOMES+CXCR6+ T cells being recruited into intrahepatic tumours due to their expansion in the pre-cancerous NASH hepatic microenvironment. This suggests that the NASH hepatic microenvironment is the key determinant to treatment response in NASH-HCC.

Figure:


1a
Hep 53.4 Intrahepatic Transplant
C57BL/6
anti VEGF-A/anti-PDL1
or IgG control
-d84
d0
d14
d28
Control Diet
or
Western Diet
Harvest
anti-PDL1: 2x weekly i.p
anti-VEGF-A 2x weekly i.p
IgG: 2x weekly i.p
1b
Intrahepatic Tumour Burden
Tumour burden / mm3
0.0021
0.1044
Lean , IgG
Lean, PDL1/VEGF
NASH, IgG
NASH, PDL1/VEGF
1c
IgG
PDL1/VEGF
Lean
NASH
1d
Intrahepatic Tumour CD8
% of CD45
0.0066
0.0146
1e
Intrahepatic Tumour
CD8+PD1+EOMES+CXCR6+
% of CD3
0.9277
0.0059
2a
Hep 53.4 Subcutaneous
C57BL/6
anti VEGF-A/anti-PDL1
or IgG control
-d84
d0
d10
d22
Control Diet
or
Western Diet
Harvest
anti-PDL1: 2x weekly i.p
anti-VEGF-A 2x weekly i.p
IgG: 2x weekly i.p
2b
Subcutaneous Tumour Burden
Tumour burden / mm3
0.0002
0.0004
2c
IgG
PDL1/VEGF
Lean
NASH
2d
Subcutaneous Tumour CD8
% of CD45
0.0031
0.3747
2e
Subcutaneous Tumour CD8 PD1+
% of CD3
0.9992
0.4232

P06-06
Gamma-aminobutyric acid B2 receptor as a potential therapeutic target for cholangiocarcinoma with diabetes mellitus

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Background and Aims: Diabetes mellitus (DM) is associated with the increased risk and progression of cholangiocarcinoma (CCA). Our previous transcriptomic analyses revealed that gamma-aminobutyric acid (GABA) B2 receptor (GABBR2) was among the top 5 upregulated genes in CCA cells cultured in high glucose conditions. The present study aimed to verify whether GABBR2 was upregulated in tumor tissues of patients with CCA. The potential of using GABBR2 as a therapeutic target was also investigated.

Method: GABBR2 expressions were determined in CCA cell lines by RT-qPCR, Western blots, and immunocytofluoresence and in tissues by immunohistochemistry. Effects of baclofen, a GABA-B receptor agonist, on CCA proliferation and colony formation were investigated by using MTT and clonogenic assays. Signaling pathways under the regulation of GABBR2 in CCA cells were screened using phospho-kinase arrays and validated in individual CCA cell lines by Western blot. All protocols of the study were approved by The Khon Kaen University Ethics Committee in Human Research (HE641441).

Results: GABBR2 was upregulated in CCA cells cultured in high glucose compared with normal glucose conditions, and the expression of GABBR2 was dose-dependent on glucose concentrations. CCA tissues from patients with DM and hyperglycemia showed upregulated GABBR2 compared to those from patients without DM. Expression of GABBR2 was associated with a poor histological type of CCA. Conversely, high GABBR2 expression was associated with smaller tumor size. Treatment of CCA cells with baclofen, a GABA-B receptor agonist, showed potent antiproliferative and anti-clonogenic effects. The antitumor effects of baclofen on CCA cells were to inhibit multiple signaling pathways, including glycogen synthase kinase 3, beta-catenin, and signal transducer and activator of transcription 3.

Conclusion: GABBR2 is upregulated in under hyperglycemic conditions in patients with CCA and DM. It is also a promising target for the improvement of CCA treatment outcomes.

P06-07-YI
Pediatric liver cancer: Hepatoblastoma and Neddylation post-translational modification

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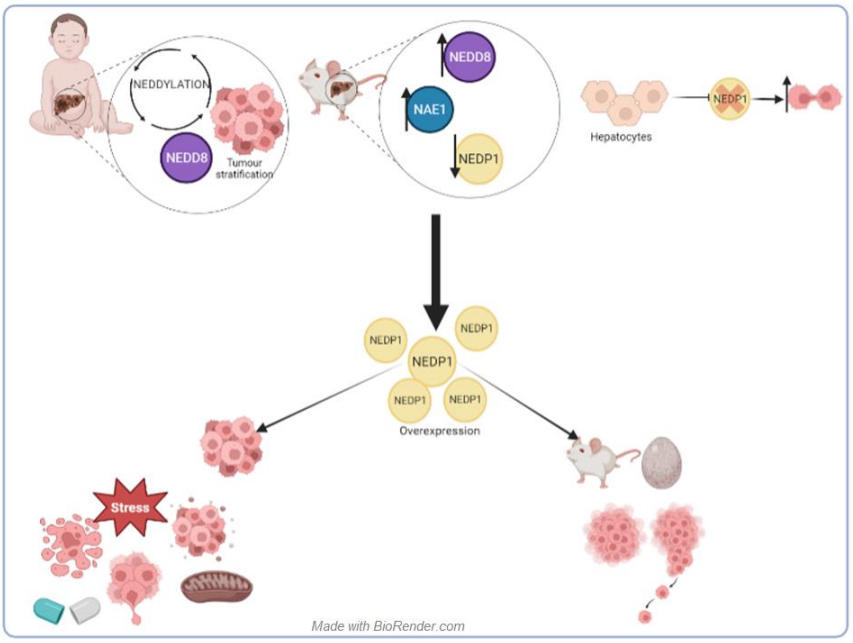
Background and Aims: Hepatoblastoma (HB), although a rare disease, is the most common form of childhood primary liver cancer. Current therapeutic options are limited or inadequate and involve significant side effects. Ongoing research has uncovered molecular, genetic, and epigenetic mechanisms that have expanded the understanding of HB, but it is an open field with many unknowns. The identification of neddylation, a post-translational modification regulated by NEDD8, widely involved in signaling pathways and in modulation of protein homeostasis, as a mechanism associated with the development of HB, has opened the doors to new therapeutic strategies. In this sense, the implications of this post-translational modification in the tumor context of HB and its modulation as a potential therapy was evaluated.

Method: A cohort of HB patients, preclinical animal model and in vitro model in tumor cells were used to characterize NEDDylation pathway in HB. Besides the modulation of NEDP1 levels using an in vitro approach was made to study cell proliferation, cell cycle, drug resistance, proteome homeostasis, and metabolic status. In vivo, the implications of NEDP1 overexpression in metastatic capability and its tumor suppressor capacity was evaluated.

Results: Transcriptomic analysis of HB patient samples has demonstrated modulation of the neddylation cycle, and NEDD8 levels correlate with the degree of tumor stratification. Preclinical models of HB, as well as the vitro models shows an increase in NEDD8 and NAE1, in addition to a significant reduction of NEDP1, demonstrating the importance of neddylation in the development and progression of this pathology. Moreover, NEDP1 silencing in human hepatocytes results in a proliferative phenotype. In contrast, its overexpression in HB tumor lines (HepT1 and HepG2) results in the induction of apoptosis, modulation of migratory and proliferative capacity, metabolic reprogramming, sensitization to chemotherapeutic treatments, and regulation of cellular stress mechanisms and immune and inflammatory responses, with modulation of proteins such as LIN28. Similarly, its overexpression in patient-derived xenografts (PDX) from a distal metastasis showed modulation of proliferation and migration, and a metabolic reprogramming like the observed in HB tumor lines. In vivo, in Ovo and ex Ovo experiments showed reduced tumorigenicity and decreased metastatic phenotype, and NEDP1 overexpression in animal models of HB in mice showed a reduction in proliferation and tumorigenesis, with modulation of proteins like HOOK2.

Conclusion: Therefore, it is noteworthy that the effect observed with NEDP1 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:



P06-15
Fascin-1 as a druggable target in agressive hepatoblastoma

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Background and Aims: Hepatoblastoma (HB) is a liver tumor that arises in children. It's a sporadic malignancy that is often very aggressive. The current treatment consists of chemotherapy. However, chemotherapy in young patients has disastrous and long-term side effects such as ototoxicity, cardiomyopathy and infertility. Thus, alternative strategies are needed. One hint is to target the most common mutations in HB. It has been demonstrated that 90% of HB tumors are mutated for the Wnt pathway effector ß-catenin. This mutation leads to an aberrant constitutive activation of Wnt/ß-catenin signaling. However, β-catenin is an essential protein and is not a druggable target. Here, we investigate one of ß-catenin targets, Fascin-1 that is found up-regulated in many tumors. Fascin1 affects actin organization into bundles and this leads to cell migration and invasion. Whereas Fascin-1 is absent from normal hepatocytes, we found its expression associated to the poor prognosis C2 subtype of HB. In both human and murine HB samples, Fascin-1 is associated to undifferentiated tumor cells. We further demonstrated that Fascin-1 expression modulates tumor hepatocyte differentiation status through gene expression. In this study, we investigate how Fascin-1 is able to regulate tumor cell plasticity and whether Fascin-1 is a druggable target in HB tumors.

Method: We use two classical HB model cells Huh6 and HepG2 and 3 Patient-Derived-Xenograft cell lines. We explore the effect of Fascin-1 actin-binding activity impairment by using inhibitors NPG2044 and BDP13176, on invasion and migration using Trans-well and wound-healing assays. We follow proliferation and cell death by Flow cytometry and investigate gene expression by PCR and reporter assay.

Results: We show that the inhibition of Fascin actin-binding activity decreases cell invasion and migration as well as proliferation. We show an increase of cell death in Huh6/HepG2 cells but not in the PDX models. Differentiation genes are overexpressed and EMT genes are repressed. Yap expression, is downregulated; Yap promoter activity is downregulated and Yap is found translocated into the cytoplasm upon Fascin-1 inhibition. These data suggest that Fascin inhibition effects on cells are mediated via the Hippo pathway.

Conclusion: Fascin-1 is an interesting target in hepatoblastoma, commercialized phase-2 drugs are available and this study will confirm the potential use of those drugs in HB treatment and elucidate by which mechanism Fascin-1 inhibition impacts tumors.

P06-16
Glycogen as metabolic fuel for tumor development in glycogen storage disease type III

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Background and Aims: Glucose represents the major source of cell energy and also contributes to provide carbon skeletons for cell proliferation. In the liver, the excess of glucose is accumulated as glycogen, and glycogenolysis is the faster process to tune blood glucose between meals. Generally, tumour cells show a metabolic reprogramming from mitochondrial respiration to glycolysis to sustain the higher growth rate. Interestingly, it has been recently described that glycogen can also support cancer cell survival under metabolic stress. However, the role of glycogen in tumour development needs to be further explored.

Glycogen storage disease type III (GSDIII) is a rare disorder caused by the lack of the glycogen debranching enzyme (GDE, encoded by the *AGL* gene), which leads to dextrin (less branched glycogen) accumulation in hepatocytes, and results into hepatomegaly and hypoglycaemia. Moreover, GSDIII patients develop hepatic fibrosis and, at a later stage, hepatocellular carcinoma (HCC).
The aim of this work was to characterize the hepatic metabolism that promotes tumour growth in GSDIII.

Method: Liver samples of $Agl^{-/-}$ mice and WT mice were analysed at the age of 14 months. Histological analyses and transmission electron microscopy (TEM) were performed to evaluate fibrosis, glycogen, and lipids. Levels of triglycerides, cholesterol, glycogen and lactate were determined by biochemical assays. Transaminase activities and alpha-fetoprotein (AFP) levels were determined from plasmas. The main metabolic and cell death pathways were analysed by RT-qPCR and western blots.

Results: As observed in GSDIII patients, $Agl^{-/-}$ mice showed hepatic glycogen accumulation, associated with hepatic fibrosis and increased transaminases compared to WT mice. Plasma AFP levels were also increased, in accordance with HCC development observed in more than 30% of 14-month-old GSDIII mice. Reduced lipid content was observed in $Agl^{-/-}$ mice. Moreover, lactate levels were drastically decreased, indicating reduced glycolysis in GSDIII livers, in accordance with low levels of hepatic glucose-6 phosphate. Importantly, tumours showed reduced glycogen accumulation compared to surrounding non-tumoral tissue and increased glycophagic flux, explained by increased lysosomal acid glycosidase activity and reduced STBD1 protein levels, the specific cargo that binds glycogen and drives it to the autophagosome during glycophagy.

Conclusion: In GSDIII, HCC development occurs in a context of fibrosis without steatosis. Interestingly, GSDIII livers showed a general energy deficiency status with low lipids and glucose levels. Our data strongly suggest that glycogen accumulation in hepatocytes might be the fuel used to promote tumour growth, particularly through the activation of the lysosomal degradation pathway in tumours.

P06-17
Fatty acid synthase promotes the malignant features of cholangiocarcinoma cells and predicts survival in patients

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Background and Aims: Cancer cells are exposed to a metabolically challenging environment with scarce availability of nutrients, and alterations in lipid metabolism may affect the cellular response to drugs. We hypothesize that fatty acids (FA) modulate the biology of cholangiocarcinoma (CCA) cells and the development of stemness features.

Method: CCA cells (HuCCT-1 or CCLP1) were treated with monounsaturated FAs (132μM oleic or 100μM palmitoleic acid). Self-renewal ability was tested with a colony formation assay. Cancer stem cell- (CSC)-enriched spheres were obtained growing cells in anchorage-independent conditions and selective medium. Five-year overall survival (OS) was analyzed in 34 patients with CCA sub-grouped based on fatty acid synthase (FASN) expression. Desaturation index and triglyceride de novo synthesis were performed by lipidomic analysis. NSG mice were injected with CCLP1 spheres and treated with the FASN inhibitor orlistat (240mg/Kg). Tumor volume was measured with Vevo LAZR-X imaging station. RTPCR array on tumor masses was performed using the QuantiNova LNA PCR Panel.

Results: Exposure of CCA cell lines to FAs increased cell proliferation and activated growth and survival pathways, including AKT and ERK1/2. Exposure to FA made CCA cells less sensitive to the toxic effects of chemotherapeutic agents, and modulated the expression of ABC transporters involved in drug resistance. The colony forming ability of CCA cells was increased by FAs, and was associated with upregulation of genes controlling epithelial-mesenchymal transition and stemness. Expression levels of genes involved in lipid metabolism were upregulated in CSC-enriched spheres as well as the percentage of desaturated TGs. FASN inhibition by orlistat decreased cell proliferation and CSC or EMT markers. In a xenograft model of CCA, tumor volume was significantly lower in mice treated with orlistat. Accordingly, expression of genes involved in cell proliferation was downregulated while the one of tumor suppressor genes increased. In a series of CCA patients, the expression of FASN correlated with OS.

Conclusion: FA promote malignant features of CCA and increase CSC markers. FASN expression levels correlate with survival in patients with CCA and promote CCA growth in mice. Lipid metabolism could be a new target to block CCA progression.

P06-19
β-neurexin expressions on hepatocellular carcinoma is associated with their increase carcinogenicity and delayed via Neuroligin-treated peptide

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Background and Aims: Hepatocellular carcinoma (HCC), the most common type of primary liver cancer, remains the third leading cause of tumor-related mortality worldwide. Several treatment options available for HCC patients comprise of surgical, liver directed and systemic therapies. However, due to late diagnosis, the surgical and liver directed approaches are usually not applicable to majority of the HCC patients. In the current study we assessed for a new marker; Neurexins, found by our lab to be expressed by HCC. Neurexins are presynaptic cell-adhesion molecules implicated in autism and schizophrenia. Neurexins are engage in trans-synaptic interactions with multifarious postsynaptic ligands, including neuroligins (NLs). Herein, we developed a newly synthesized NL-4 peptide (amino acid based) to assess their effects on HCC activities in an in vitro culture model.

Method: HCC cell-line (Hep3B) showed 95% expressions of b-neurexin through the flow cytometry, western blot, and RT-PCR analysis. Gene expressions of cell cycle (Cyclin dependent kinase inhibitor 2A (CDNK2A)) and proliferation (MKI67 and platelet-derived growth factor receptor α (PDGFA) following NL-4 peptide treatments (8 mg/ml) were performed. Assessments of HCC cell cycle alterations and apoptosis were also assessed. Signalling pathways of phosphorylated p53, PI3K, AKT and mTOR were also evaluated.

Results: Cancer makers of phosphorylated p53 and AKT/PI3K/mTOR signalling pathway were diminished with less proliferative cells with reduced PDGFRA gene expressions following NL-4 peptide treatments. These results were in line with delay in G2-M phase of Hep3B to 1.39-folds with a significant decrease in the S phase was seen Moreover, data demonstrated a reduced Hep3B necrosis to 2.1-fold ($P<0.01$) associated with inhibited gene expressions of CDNK2A.

Conclusion: These data show significant disturbance in cell cycle parameters in the G2/M phase (mitosis state) along with a significant shifting to the G1 phase (naive state), indicating a marked delay in the mitotic phase following NL-4 peptide suggesting anti-cancer and anti-proliferative effects and could be a promising future cancer remedy.

P07-02-YI
Role of SerpinB3-PD polymorphism in the prognosis of hepatocellular carcinoma

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Background and Aims: Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide. Although curative options can afford long-term survival in patients with early-stage tumours, the actual strategy for HCC surveillance misses over one-third of HCC at an early stage. Chronic inflammation and neoangiogenesis play a central role in the promotion of carcinogenesis. SerpinB3 (SB3) is a serine proteinase inhibitor involved in tumorigenesis and in the promotion of a pro-inflammatory milieu. The polymorphic variant SerpinB3-PD (SB3-PD) presents a substitution in the reactive center loop of the protein (Gly351Ala), determining an improved anti-protease activity. This study aims to investigate the role of SB3-PD as a predictor of mortality in patients with liver cirrhosis and HCC and to assess the effect of SB3-PD in the pro-inflammatory response in vitro.

Method: SB3-PD polymorphism was assessed in 107 cirrhotic patients with HCC, followed up from HCC diagnosis to death or transplant for a median period of 21 months. The results were analyzed in relation to clinical, radiological, laboratory data, BCLC stage at baseline, and treatments of HCC during follow-up. In vitro study was conducted on human monocytic leukaemia cell line (THP-1) treated with recombinant protein SB3-wild type (WT) or the polymorphic variant SB3-PD. mRNA quantification of pro-inflammatory cytokines was performed by quantitative real-time PCR, whereas protein quantification was carried out by ELISA.

Results: Patients carrying SB3-PD polymorphism (n=38; 35.5%) at diagnosis showed larger tumours, higher alpha-fetoprotein levels and more frequent vascular invasion and metastasis, associated to a significantly lower survival compared with patients carrying SB3-WT (34.3 vs 53.8 months, p=0.009). SB3-PD was proven to be an independent predictor of mortality in patients with HCC, together with BCLC stage, ALBI grade, alpha-fetoprotein values, N/L rate and ALP values. In vitro results showed that THP-1 cells treated with SB3-PD had a more abundant and prolonged cytokine production, compared to controls

Conclusion: Patients with liver cirrhosis and HCC carrying SB3-PD polymorphism have a poorer prognosis and the SB3-PD variant determines a stronger pro-inflammatory response, supporting its greater carcinogenetic effect. The assessment of SB3-PD polymorphism could help to personalize screening strategies for HCC.

P07-03-YI
Identification of hepatocyte-restricted antigens, epitopes, and T cell receptors to treat recurrent hepatocellular carcinoma after liver transplantation

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YSR and DK contributed equally to this research project.
SIB, RD, and DS contributed equally to this research project.
Target antigens, epitopes, and T cell receptors are not disclosed due to patent filing

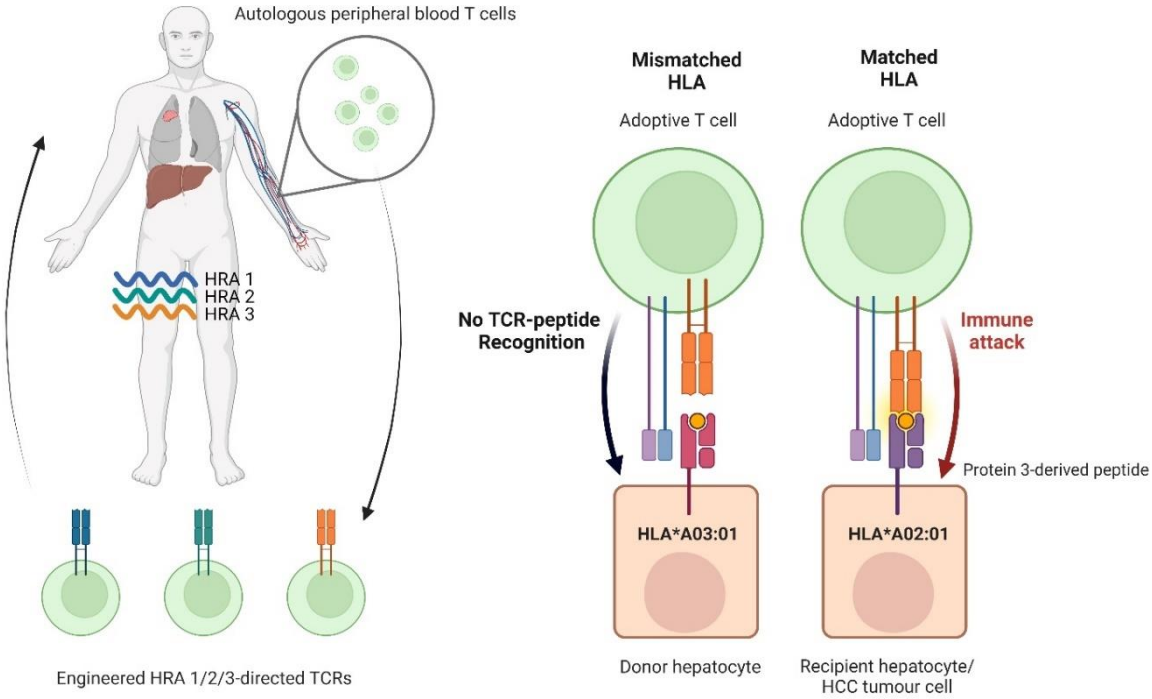
Background and Aims: HCC recurrence in the context of an HLA-mismatched donor liver provides the unique setting that liver antigens from HCC versus the liver allograft are presented by different alleles of Human Leukocyte Antigen (HLA). Here, we present the development of an adoptive therapy with T cell receptor (TCR)-engineered T cells directed against hepatocyte-restricted antigens (HRAs) presented by the recipient, but not donor HLA.

Method: We have applied an integrative approach of *in silico* antigen and epitope prediction, immunopeptidomics, and *in vitro* laboratory tools to stringently select and validate HRAs, their immunogenic epitopes, as well as corresponding TCRs.

Results: 58 presumed liver antigens retrieved from the human protein ATLAS were further evaluated for liver-restricted expression in 6 public RNA databases and 1 protein database (HIPED), shortlisting 14 candidate HRAs. 3/14 HRAs did not show RNA expression in healthy tissues, except for liver, in another five tissue datasets (n=1,709) and validated using qPCR. Two HRAs demonstrated RNA expression in >70% of HCC patients (n=421). Immunopeptidomics of HCC-derived hepatocytes (n=12), together with *in silico* predictions of immunogenicity, revealed 36 HLA-A2-restricted epitopes. These epitopes were tested and ranked according to *in vitro* HLA-A2 binding ability. Epitope-specific T cells were enriched from healthy donors for 6 of these epitopes using an *in vitro* co-culture with autologous antigen presenting cells. Eleven TCRαβs directed against 4 HRA-derived epitopes were selected following epitope-MHC-directed fluorescence-activated sorting of T cells. Five TCRs were functionally expressed upon gene transfer into T cells and recognized their cognate peptide, of which 4 TCRs harboured a stringent safety profile according to amino acid scanning, and are expected to mediate no to negligible cross-reactivity.

Conclusion: We have identified HRAs, epitopes and corresponding TCRs, of which the lead TCRs will be further exploited for the treatment of recurrent HCC after liver transplantation with adoptive therapy of TCR-engineered T cells.

Figure:



Concept of TCR-engineered T cells to exclusively target recipient-derived hepatocytes and hepatocellular carcinoma. In the current study, hepatocyte-restricted antigens (HRA) and their epitopes are identified following which HRA-specific T cell receptors (TCRs) are obtained with reactivity towards hepatocytes/HCC tumour cells but not to donor hepatocytes. These TCRs, when used to gene-engineer autologous, peripheral blood T cells, constitute a therapeutic to treat HCC recurrence after liver transplantation leaving the liver allograft unaffected.

P07-04-YI
Fibroblast growth factor 21 delays hepatocellular carcinoma development and is critical for liver regeneration in a murine model of moderate and severe liver injury

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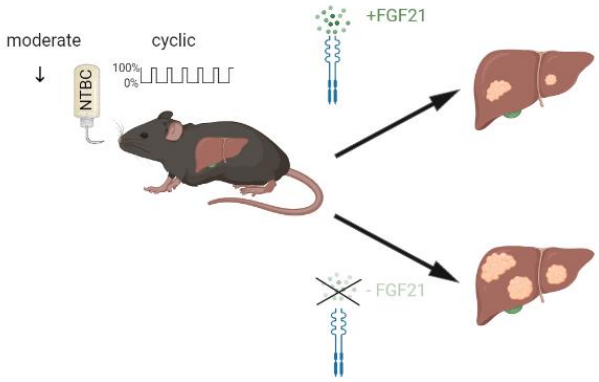
Background and Aims: Hepatocellular carcinoma (HCC) is the most common primary liver cancer and a leading cause of cancer-related deaths worldwide. A better molecular understanding of the development of HCC will be critical to identify potential biomarkers for cancer therapies. Recent data provide evidence that fibroblast growth factor 21 (FGF21), mainly expressed in the liver, is an emerging therapeutic target for non-alcoholic fatty-liver disease (NASH), and loss of FGF21 promotes tumorigenesis in NASH models. Here, we aimed to investigate the role of FGF21 in liver damage, regeneration and hepatocarcinogenesis in a non-metabolic liver injury model.

Method: The *Fah* knockout mouse was crossbred to *FGF21*$^{-/-}$ mice. *Fah*$^{-/-}$ mice develop liver injury and HCC due to the accumulation of the toxic metabolite fumarylacetoacetate, which can be prevented by treatment with nitisinone (NTBC). Liver tissues of *Fah/FGF21*$^{-/-}$ and the respective *Fah*$^{-/-}$ controls that received either a cyclic treatment or reduced dose of NTBC were examined macroscopically and histologically and compared to healthy livers (100 % NTBC). In the *Fah* deficient background, H&E, Sirius Red and Oil Red stainings were performed to determine tumor penetrance and the degree of liver damage. This result was validated by liver transaminases as biochemical marker of increased liver injury. Liver weight and Ki67 expression were used as surrogates for the regenerative capacity.

Results: Tumor onset was accelerated and tumor penetrance was higher in the absence of FGF21 in the setting of moderate liver injury mediated by reduced NTBC dose, although liver injury was not increased. In mice with severe liver injury mediated by cyclic NTBC treatment, FGF21 loss similarly accelerated carcinogenesis, and also aggravated liver fibrosis. In acute liver injury, basal regeneration rate of *Fah/FGF21*$^{-/-}$ livers was significantly impaired compared to *Fah*$^{-/-}$ control mice, despite increased liver injury in these livers. Downstream signaling pathways regulated by FGF21 and immune cell distribution, which may have an impact on tumorigenesis, are currently under investigation

Conclusion: Overall, our data revealed that loss of FGF21 accelerates hepatocarcinogenesis in the setting of moderate and severe liver injury in the *Fah* model and even exacerbates fibrosis in the latter. Moreover, FGF21 was found to play a critical role in the regenerative capacity of livers after acute liver injury. Recently published phase II clinical data showed that an FGF21 analogue, such as efruxifermin, significantly improved a number of metabolic and inflammatory parameters in patients with NASH. Our data suggest that FGF21 may also be an interesting chemopreventive agent for patients suffering from non-metabolic liver diseases.

Figure:



P07-05
The earliest integration of hepatitis B virus into hepatocyte genome: sites, mechanism and role in HCC oncogenesis

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Background and Aims: More than 50% of newly diagnosed HCC coincides with hepatitis B virus (HBV) infection. Integration of HBV DNA into hepatocyte genome invariably precedes HCC development and plays a pivotal role in HBV oncogenesis. We aimed at identification of the initial (earliest) sites of HBV integration, kinetics and mechanism of their formation, and recognition of their role in HCC initiation.

Method: Hepatocytes and hepatocyte-like cells *de novo* infected with authentic HBV or HBV-closely related woodchucks hepatitis virus (WHV) and naïve woodchucks infected with wild-type WHV were analyzed from 15 min to 72 h post-infection (p.i.). Virus-host genomic junctions were detected by inverse-PCR, verified by virus nucleic acid hybridization (NAH), identified by clonal sequencing, and mapped by computer-assisted programs.

Results: First virus-host DNA fusions occurred within 15 min p.i. The majority were head-to-tail joints (HTJs) implying formation by the non-homologous end joining (NHEJ). Retrotransposons and genes with translocation potential were frequent HBV targets. Other host genes were also identified as early insertional sites in human and woodchuck hepatocytes and in livers of woodchucks infected with WHV. Formation of the earliest virus-host DNA junctions coincided with strong induction of reactive oxygen species (ROS), hepatocyte DNA damage, and activation of the poly(ADP-ribose) polymerase 1 (PARP1)-mediated DNA repair machinery. The PARP1 engagement was accompanied by augmented transcription of XRCC1 (the PARP1 binding partner) and OGG1 (a responder to oxidative DNA damage), and by increased activity of NAD^+ (a marker of PARP1 activation) and HO1 (an indicator of cell pro-oxidative stress response).

Conclusions: HBV and WHV integrate into hepatocyte genome almost instantly after virus invasion before molecular evidence of viral replication is detectable. Both viruses are potent oxidative stress inducers causing immediate hepatocellular DNA damage and activation of the PARP1-mediated DNA repair machinery. Engagement of this DNA repair pathway explains predominant HTJ configuration of the initial HBV-host genomic fusions. Retrotransposons and genes with translocation potential are common among initial sites of HBV insertion suggesting a mechanism for spread of virus DNA across hepatocyte genome from the earliest stages of infection.

P07-06-YI
KDM2A histone demethylase ablation restores immunogenicity in liver cancer

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Background and Aims: Genetic information is packaged into chromatin and accumulating evidence suggest that modulations of chromatin structure such as histones assume a relevant significance in genomic integrity. However, how the chromatin remodelers intervene in maintaining genomic integrity and how they affect genetic information remain poorly understood. Here we report that ablation of KDM2A induces DNA damage/DNA damage response (DDR), mitochondrial dysfunction, and triggers an immune response. KDM2A specifically removes mono- and di-methyl moieties from lysine 36 of histone H3 (H3K36). It has been implicated in the regulation of CpG islands and as chromatin-modifying enzyme promotes transcriptional activation or repression.

Method: Human HCC cell lines (HepG2, Huh7), mouse HCC cell line (Hepa1-6) and hTert immortalized human hepatocytes (Hus) were employed. To assess the functional role of KDM2A, gain on-and loss-of-function, chromatin immunoprecipitation, RNA-seq, immunofluorescence/live imaging was performed. Mitochondria Oxphos was evaluated by measuring O_2 consumption and mitochondrial membrane potential by TMRM staining, H_2O_2 was measured by colorimetric assay. Hepa 1-6 cells depleted of KDM2A by using CRISPR/Cas9 were further analyzed in in vitro and in vivo experiments.

Results: To identify chromatin regulators of genomic stability, knock down of checkpoint kinase 2 (CHK2) a central effector of DDR and DNA repair was performed, and transcriptomics was analysed.Gene Ontology and STRING analysis of differentially expressed genes revealed that the upregulated genes were significantly related to interferon-stimulated genes (ISGs). Importantly, Huh7 cells that did not reveal modifications in ISGs expressed high levels of KDM2A. To explore the biological function of KDM2A we depleted KDM2A with RNAi in Huh7, HepG2 and Hepa1-6 and all cells tested showed increased expression of phospho-histone H2AX a marker of DNA damage. Moreover, Huh7, HepG2, and Hepa1-6 cells showed activation of DDR pathway. To unravel the source of DNA damage we hypothesized an involvement of mitochondria. Indeed, mitochondria of KDM2A depleted cells showed a defective efficiency of Oxphos associated with increased production of H_2O_2. Important,chromatin modified by depletion of KDM2A led to re-expression in cancer cells of adaptive immunity proteins PD-L1 and HLA-DRB1, activation of IFI16-STING signaling, and cell growth inhibition in vitro and in vivo. Consistently, TGCA data analysis showed KDM2A overexpression in liver cancer and inverse correlation between KDM2A expression and T cell infiltration.

Conclusion: Our findings suggest a role of KDM2A as epigenetic controller of genome stability and immunogenicity in liver cancer. Along these lines targeting KDM2A by siRNA-GalNAc conjugated combined with PD-L1 blockade could represent a novel cancer treatment strategy.

P07-14
Hepatocellular Carcinoma cell lines growth inhibition by liver-derived mensenchymal stem cells in direct 3D co-culture

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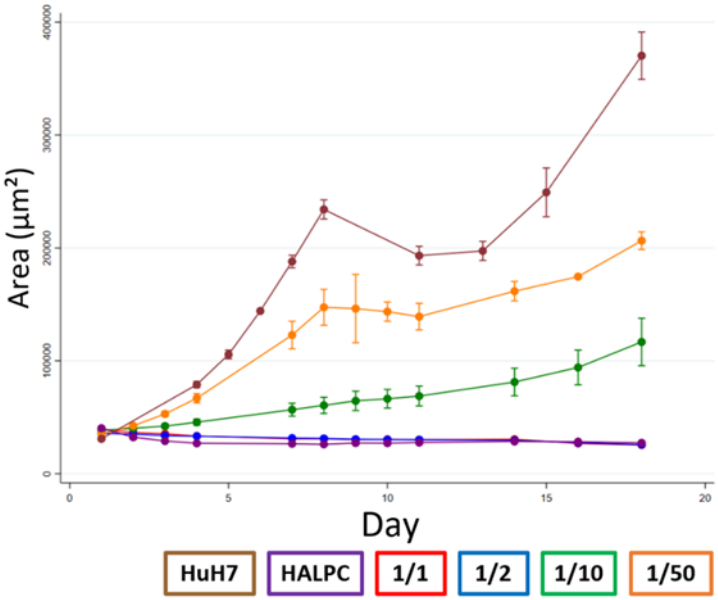
Background and Aims: Hepatocellular Carcinoma (HCC) is the second highest cause of cancer-related death. Current systemic therapeutic approaches are unable to offer long-term survival perspectives to patients. Recently, cell therapy using mesenchymal stem cells (MSCs) is being explored as a potential new therapeutic strategy in oncology. MSCs display innate anti-tumoral properties in various *in vivo* and *in vitro* models but their exact role in tumor emergence and progression is still highly debated, as other studies also highlighted pro-tumoral effects. Human Adult Liver Progenitor Cells (HALPCs), a liver-derived mesenchymal-like population of stem cells, are being developed as allogeneic products. The aim of this work is to investigate the therapeutic properties of HALPCs in the context of HCC through the study of their interactions with three human HCC cell lines (HepG2, Hep3B and HuH7) in an *in vitro* 3D co-culture model.

Method: Mono-cellular and bi-cellular spheroids were formed in BIOFLOAT (faCellitate) ultra-low attachment plates by seeding 1000 cells/well (10 000 cells/well for histological analyses). In bi-cellular spheroids, HALPCs and either HepG2, Hep3B or HuH7 cells were seeded at different cell-to-cell ratios to explore spheroid growth by daily brightfield micrography. Total spheroid area measurement was automated using an ImageJ Macro. Cell proliferation was assessed through luminometry measurement of the total ATP content of spheroids using the Cell-Titer Glo 3D kit (Promega) and Ki67 immunostaining on paraffin-embedded spheroid slices.

Results: The spheroids' size follow-up revealed a statistically significant decrease in spheroid growth velocity when HALPCs were co-cultured with the different cell lines. This reduced growth of HCC cells was more pronounced with Hep3B and HuH7 than with HepG2. This effect was also dependent on the HALPC ratio in the spheroid. Biochemical and histological analyses supported those results, with a significant decrease of the total ATP content and the number of Ki67 + cells in bi-cellular spheroids when compared to their respective controls.

Conclusion: In a 3D *in vitro* direct co-culture model, HALPCs are able to reduce the proliferation of three different human HCC cell lines (HepG2, Hep3B and HuH7). This effect was proven morphologically, biochemically and histologically. The deciphering of the mechanisms of action of these effects is ongoing while *in vivo* validation of these results is mandatory.

Figure:



HuH7 and HALPC bi-cellular spheroids growth follow-up

Bi-cellular spheroids were formed in BIOFLOAT plates (*faCellitate*) by seeding 1000 cells/well at varying ratios of both cells types (1/1 consists of 500 HALPC and 500 HuH7, 1/2 consists of 333 HALPC and 666 HuH7, ...). Mono-cellular spheroids were used as controls (HuH7, HALPC). The graph shows the daily mean area of each subtype of spheroid with their confidence interval (n = 32).

P07-16
Establishment and characterization of a patient derived xenograft model of cholangiocarcinoma

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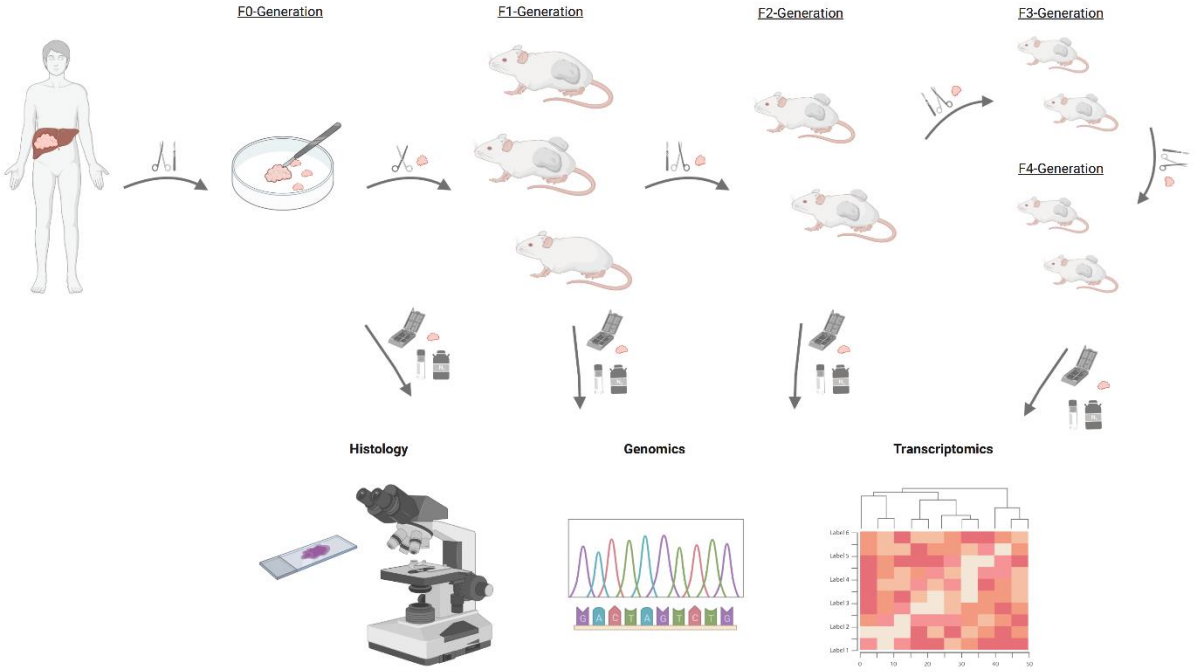
Background and Aims: Cholangiocarcinoma (CCA) is the second common type of liver cancer. 5-year-survival remains below 10 %, mainly caused by the aggressive biology, late diagnosis and lack of curative therapies. In order to develop more effective therapies, there is an urgent need for a better molecular understanding of CCA as well as for appropriate preclinical models for drug testing. Patient derived xenografts (PDX) serve as valuable tools to mimic physiologic conditions *in vivo*. Here, we generated and characterized PDX models of CCA.

Method: Resected patient tumors (n = 15) were subcutaneously implanted in immunodeficient mice, and monitored for tumor growth. Successfully engrafted tumors were passaged for up to four generations; tumor tissue was subjected to histologic evaluation and to molecular characterization by DNA as well as RNA sequencing.

Results: PDXs were established with a success rate of ~ 50 %, independent of patient gender or age. Latency varied between 39 and 280 days. Upon re-transplantation of PDX tumor tissue, latency was significantly accelerated and penetrance was nearly complete across up to three subsequent transplantations. Histologically, PDX tumors overall resembled the parental tumors, but less desmoplastic stroma was observed. The morphology of replicate PDXs upon re-transplantation was highly comparable. Based on transcriptome analysis, parental tumors and the respective PDXs clustered closely together, and stromal signatures were longitudinally downregulated upon *in vivo* passaging.

Conclusion: We established and serially passaged seven PDXs from patients with intrahepatic CCA. PDXs retained key morphological and molecular characteristics of the original patient tumor. We expect, that thoroughly characterized PDX will serve as valuable tool for drug testing and to understand primary and secondary resistance mechanisms to targeted therapies.

Figure:


F0-Generation
F1-Generation
F2-Generation
F3-Generation
F4-Generation
Histology
Genomics
Transcriptomics

P07-18
Programmable DNA hydrogel assisting microcrystal formulations for sustained locoregional drug delivery in residual tumor foci

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Background and Aims: Adverse events caused by systemic administration of antitumor agents commonly lead to the discontinuation of therapies in patients with carcinoma. For treatment of residual tumor foci with rare vascular, locoregional continuous drug action is required. However, it is challenging to develop a sustained drug delivery system for the locoregional treatment of cancer, e.g., hepatocellular carcinoma (HCC).

Method: The DNA strands used in this study were all synthesized by Shanghai Sangong Biotech Co. Ltd and purified by HPLC. To prepare DNA oligonucleotides for gelation, H1 and H2 were heated at 95℃ for 5min and then quickly quenched to 4℃ in ice for annealing. To make blank or drug-loaded DNA hydrogel samples, H1, H2 and initiator I were dissolved in 1×TAE-Mg2+ buffer (40mM Tris, 20mM acetic acid, 2mM EDTA, 12.5mM Mg2+, pH=8.0) with or without the drug Elimusertib, the mixture was then transferred into syringes so that hydrogels could be taken out en bloc for further characterization tests.

Results: Here, we report a drug-laden hydrogel generated from pure DNA strands and highly programmable in adjusting its mesh size. Meanwhile, the DNA hydrogel could assist the micro-crystallization of a novel ATR inhibitor, Elimusertib, further facilitating its long-term release. When applied to the tumor site, the hydrogel system demonstrated significant antitumor activity, minimized systemic toxicity, and had a modulatory effect on the tumor-immune cell interface.

Conclusion: Our drug-loaded DNA-hydrogel platform represents a novel modality for (neo)adjuvant therapy in patients with HCC.

P08-03
Effect of Zinc Acexamate treatment in hepatocellular carcinoma in vitro and in vivo models

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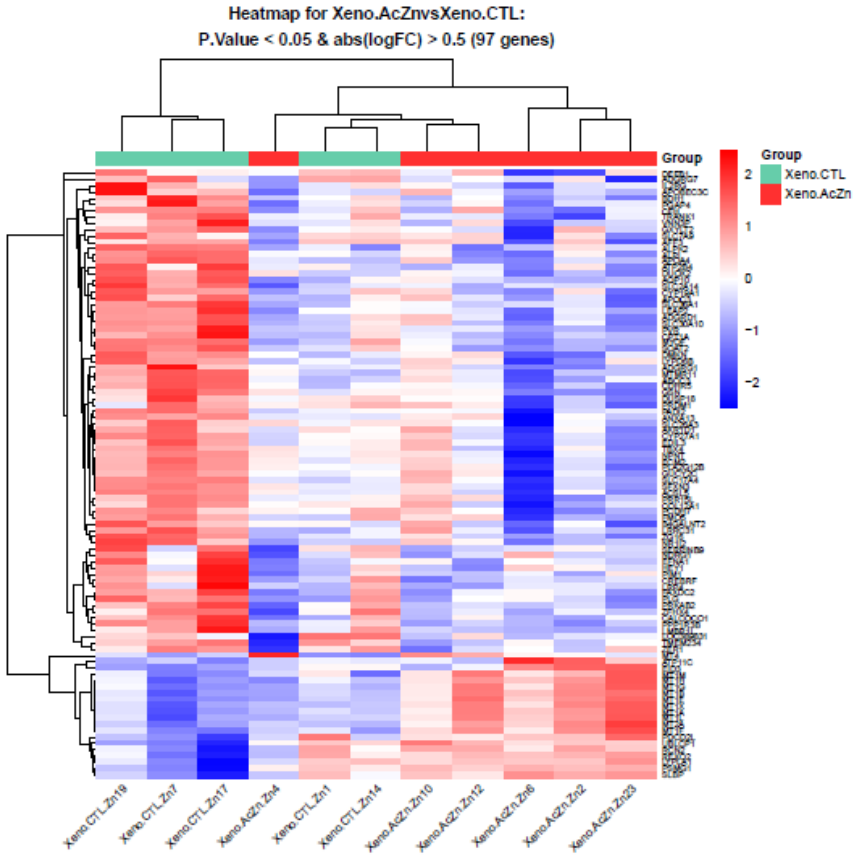
Background and Aims: Hepatocellular carcinoma (HCC) is the leading cause of death in patients with cirrhosis. Zinc (Zn) is an essential element involved in several cellular processes and its deficiency has been observed in patients with liver cirrhosis and HCC. Zinc acexamate (ZnAc) is an approved and commercialized drug for peptic ulcer treatment whose activity is based on its Zn content. Our objective was to evaluate the effect of ZnAc supplementation in an in vitro and subsequently in an in vivo model of HCC.

Method: Four HCC cell lines (HepG2, Hep3B, SNU423 and HuH7) were treated with different concentrations of ZnAc. Cell viability was assessed by MTT assay, colony formation, migration and invasion capacity by 8 uM membrane and wound healing assay (WHA) and cell cycle and apoptosis by flow cytometry. Subsequently, a xenograft model of HCC was used to evaluate the in vivo effect of ZnAc. After injecting 5x106 HuH7 cells at the right flank of 24 nude mice, they were divided into 4 different groups of treatment: control, ZnAc, Sorafenib and ZnAc+Sorafenib. Tumor growth was monitored and when a size of 1500mm3 was reached mice were euthanized. Upon euthanasia, tumor tissue was removed to evaluate the differences in gene expression between the control and the ZnAc groups by microarrays.

Results: Cell viability showed a dose-dependent response after 24, 48 and 72h of ZnAc treatment, the median LC50 was 109.3uM, 203.5uM and 50.1uM respectively. A concentration of 100uM ZnAc was used and it affected the colony formation ability, significantly decreasing it by an average of 44%, the migration ability by inserts decreased by 75% and by WHA by 58%. In contrast, no significant differences in cell cycle or apoptosis were observed after ZnAc treatment. In vivo, no significant differences were found in tumor growth of the treated mice in the different groups, although there were no signs of toxicity. Regarding gene expression, we found a different pattern of expression between the samples of the control group and those treated with ZnAc, a total of 348 genes were found to be over-expressed and 881 under-expressed in the ZnAc group compared with the control group ($p<0.05$). 97 of these genes have an absolute log fold change above 0.5 and are represented in the heatmap of figure 1.

Conclusion: HCC cell lines are sensitive to ZnAc supplementation in a dose-dependent manner. ZnAc supplementation decreases viability and colony formation and reduces the ability of HCC cell lines to migrate and invade. However, ZnAc does not significantly improve tumor progression in an in vivo model of HCC, although differences in gene expression have been found in the tumor tissue of ZnAc-treated mice compared to control. Histological studies and deeper analysis are currently ongoing.

Figure:


Heatmap for Xeno.AcZnvsXeno.CTL:
P.Value < 0.05 & abs(logFC) > 0.5 (97 genes)
Group
Group
Xeno.CTL
Xeno.AcZn
2
1
0
−1
−2
Xeno.CTL.Zn19
Xeno.CTL.Zn7
Xeno.CTL.Zn17
Xeno.AcZn.Zn4
Xeno.CTL.Zn1
Xeno.CTL.Zn14
Xeno.AcZn.Zn10
Xeno.AcZn.Zn12
Xeno.AcZn.Zn6
Xeno.AcZn.Zn2
Xeno.AcZn.Zn23

P08-04-YI
The role of Aurora kinase A in hepatocellular carcinoma: possible regulation of Programmed death-ligand 1

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Background and Aims: Aurora kinase A (AURKA) has a key mitotic role in G2/M transition, spindle assembly, and centrosome maturation. In cancer, AURKA plays relevant regulatory roles in several oncogenic pathways including the activation of Programmed death – ligand 1 (PD-L1) in breast cancer. Thus, numerous drugs targeting AURKA have been developed. Only limited information about the role of AURKA in hepatocellular carcinoma (HCC) is available. AURKA is higher expressed in HCC and associated with increased proliferation and angiogenesis, however, no evidence exists about its function in PD-L1 regulation. Therefore, we aim to explore the correlation between AURKA and PD-L1 in HCC tissues and evaluate the involvement of AURKA in PD-L1 regulation.

Method: AURKA and PD-L1 mRNA expression was evaluated by Quantitative Real-Time PCR (RT-qPCR) in 39 HCC nodules and paired distal tissues from resected livers. JHH6 cells, an undifferentiated HCC-derived *in vitro* model, were treated for 72 hours with two inhibitors of the kinase activity of AURKA (Alisertib and AK-01) and short interfering RNA (siRNA) targeting AURKA. The kinase activity of AURKA was evaluated by assessing the phosphorylation of LATS2 (Ser83). The effects of the treatments were assessed in terms of cell viability (MTT assay), cell cycle arrest (FACS), chromatin content, and centrosome changes (immunofluorescence). RNA and protein expressions were determined through RT-qPCR and Western Blot, respectively.

Results: The expression of AURKA was significantly higher in HCC nodules compared to paired-distal tissues ($p = 0.007$) with upregulation in 69% of samples and a significant positive correlation with PD-L1 expression in both nodules and distal tissues ($p < 0.001$). Using the median expression of AURKA as the cut-off value, we divided the population into High- vs Low-AURKA groups. The High-AURKA group has significantly higher levels of PD-L1 in both nodule and distal tissues ($p < 0.001$). *In vitro*, the treatments with AURKA inhibitors or siRNA significantly decreased LATS2 phosphorylation ($p < 0.001$) and cell viability ($p < 0.001$). In addition, we observed chromatin accumulation associated with an increase in centrosomes and incorrect chromosome alignment during mitosis. All treatments significantly reduced PD-L1 protein expression (Alisertib: $p = 0.026$; AK-01 and siRNA: $p < 0.001$) with major reductions caused by AK-01 at 72 hours (38%) and siRNA at 144 hours (35%).

Conclusion: AURKA is positively correlated with PD-L1 in both HCC and non-tumor tissues. The inhibition of kinase activity of AURKA enhances the number of polynucleated cells due to defects in chromosome separation and incorrect mitosis. The reduced expression of PD-L1 following AURKA inhibition highlights the potentiality of AURKA inhibitors in cancer therapy, possibly in combination with new immune checkpoint inhibitors.

P08-05
A novel hepatocellular cancer patient derived organoid xenograft model to investigate impact of liver regeneration on tumor growth

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Background and Aims: Recurrence is a significant problem following treatment for Hepatocellular Carcinoma (HCC). It affects more than 70% of patients receiving a surgical resection. Recurrence can arise from undetected micrometastasis (multicentric tumor) or “de novo” cancer. Clinical and experimental studies suggest that liver regeneration ensuing surgical resection may activate occult micro-metastasis leading to tumor recurrence. More work is required to compare the gene and protein expressions in normal and malignant liver cells in a regenerative liver environment to identify targetable signaling pathways unique to cancer cells. Ultimately unifying the opposing concepts of an anti-tumor microenvironment and beneficial conditions for liver regeneration. Here, we aim to establish an in vivo model to understand the impact of liver regeneration on HCC tumor growth.

Method: Patient Derived Organoids (PDOs) were generated from HCC tissue obtained from patients that underwent liver resection at the University Center for Gastrointestinal and Liver Diseases (Clarunis). HCC-PDO organoids have been proved to retain the histopathological characteristic of the original tumor. After a laparotomy, HCC-PDO were implanted in the liver of NOD Scid gamma mice. Successful implantation and growth were verified by sonography. Animals were randomized to control or experimental group. Experimental group undergo a re-laparotomy and 30% or 70% hepatectomy while control group only receives a re-laparotomy. Tumor growth was monitored by ultrasound until the end-point of the experiment. Healthy and tumor tissue were characterized using immunohistochemistry.

Results: The HCC-PDOs implantation process has been refined and optimized regarding scheme of anesthesia, volume and speed of injection. We have successfully performed the implantation of 2 HCC-PDO lines. Tumors obtained from the orthotopic models maintain the histopathological characteristic of the initial tumor. Preliminary results from animals that received resections after implantation, showed an increase in tumor growth compared to control. The resection group (n = 4) of organoid line 1 showed a mean increase of 256.4 percent (SD ± 416.9) compared to 45.14 percent (SD ± 9.987) mean increase in the control group (n = 2) (p = 0.536). The resection group (n = 6) of organoid line 2 showed a mean increase of 41.50 percent (SD ± 75.13) compared to 22.24 percent (SD ± 12.73) mean increase in the control group (n = 4) (p = 0.632).

Conclusion: Preliminary data shows an increased tumor growth rate in our resection groups compared to control groups. Our established orthotopic xenograft model can help understand the molecular basis of HCC recurrence after surgery. Therefore, this model may provide the basis for future projects for specific drug therapy before or after liver resection to inhibit tumor growth and favour regeneration.

P08-09
Stabilization of O-GlcNAcylation increases Enhancer of Zeste Homolog 2 (Ezh2) direct targets in hepatocellular carcinoma

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Background and Aims: Enhancer of Zeste Homolog 2 (Ezh2) is frequently upregulated in hepatocellular carcinoma (HCC) tissues and increased Ezh2 expression correlates with HCC aggressiveness and/or poor prognosis. Ezh2 knockdown in HCC cells has been shown to reverse tumorigenicity in a nude mouse model, suggesting a potential therapeutic value of Ezh2 inhibition in HCC. The histone methyltransferase Ezh2 inhibitor tazemetostat has been FDA-approved for locally advanced or metastatic epithelioid sarcoma. However, this inhibitor demonstrated low efficacy in solid tumors suggesting that histone methyltransferase independent functions of EZH2 play an important role in cancer. As the catalytic subunit of PRC2, Ezh2 is responsible for H3K27 di- and trimethylation associated with gene repression. In additional to this canonical activity, Ezh2 can also activate gene transcription. Ezh2 activity is regulated by post-translational modifications, including glycosylation by OGT. We have previously shown that OGT expression is increased in tumor tissue from HCC patients. Interestingly, it has been reported that OGT and Ezh2 co-repress a defined tumor suppressor genes in breast and colon cancer cells. The aim of our project is to assess whether OGT and EzH2 can regulate cancer-associated pathways in HCC and to determine the underlying molecular mechanisms.

Method: Paired liver tumor and peri-tumor tissue samples derived from HCC patients were analyzed by RT-PCR. Transcriptomic analysis of human hepatoma HepG2 cells following Ezh2 or OGT silencing was performed using RNA-sequencing (RNA-seq). Chromatin immunoprecipitation followed by sequencing (ChIP-seq) experiments were done using antibodies directed against OGT, Ezh2, H3K27me3 or H3K27ac.

Results: We showed that OGT and Ezh2 are upregulated in tumor tissues as compared to peri-tumor tissues in 152 HCC patients of a French cohort as well as 50 patients from the TCGA LIHC cohort. RNA-seq and ChIP-seq analysis of human hepatoma cells indicate that OGT and Ezh2 co-modulate the expression of a significant number of genes, including genes involved in cell cycle and cancer pathways. Notably, only a minority of co-regulated genes appear to be co-repressed by Ezh2 and OGT. Our data suggest that Ezh2/OGT mostly promote gene expression in liver cancer cell lines and HCC patients. Interestingly, stabilization of O-GlcNAcylation increased the number of Ezh2/OGT target genes suggesting that O-GlcNAcylation of Ezh2 and/or other co-factor(s) contributes to define the repertoire of Ezh2 targets in liver cancer.

Conclusion: Our results demonstrate that OGT plays an important role in the regulation of Ezh2-mediated gene expression in transformed liver cells. Stabilization of O-GlcNAcylation increases the repertoire of Ezh2 direct targets. Our data provide important insights for epigenetic strategies as potential future anti-HCC therapies.

P08-11
A transcriptomic signature of Intrahepatic Cholangiocellular Carcinoma with very early recurrence: Identifying patients at risk

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Background and Aims: Intrahepatic cholangiocarcinoma (ICC) is the second most common primary liver cancer with a 5-year overall survival of 25-40% and a tumor recurrence rate of 50–70%. Liver surgery and transplantation are the only curative options. Nowadays the available predictors of recurrence are not sufficient to stratify patients before surgery. In this study we seek to identify patients at high risk for very early recurrence by combining clinicopathological parameters and a gene expression signature.

Method: We will perform gene expression analysis from primary tumor tissues of patients with very early recurrence (<6 months) and a progression free survival (PFS) > 6 months. The differentially expressed gens between the groups will be used for the signature generation and then combined with other clinicopathological parameters to provide a risk-stratification model. Clinical training and validation of risk stratification model will be performed by analyzing prospectively recruited clinical cohorts.

Results: Combined databases from University Hospital of Basel, Switzerland and Humanitas University Hospital, Milan, Italy provide 12 samples with very early recurrence and 53 samples with a progression free survival > 6 months. Using currently available clinical risk scores only two of 12 patients (17%) of our cohort with very early recurrence were considered to have a high probability of very early recurrence. 10 patients (83%) of our very early recurrence cohort were predicted to have a low probability of very early recurrence by the risk score. An analysis of the patient characteristics of our cohort showed that only the microvascular invasion status was significantly associated with progression free survival.

Conclusion: Currently the practice of patient stratification into high risk of very early recurrence if practiced at all is insufficient. This seems especially daunting when considering the severity of surgery needed to cure intrahepatic Cholangiocarcinoma. Often there is a high morbidity and mortality associated with the interventions. An improved patient stratification can aid the important process of patient selection. In this case in two ways: either rethinking the decision to perform an operation with high morbidity in a patient with a very high risk of early recurrence. Or in encouraging the treatment decision in a patient with a low risk of very early recurrence.

P08-13
The gene expressions and bioinformatics analysis of miR-146b-5p and miR-4510 in Hepatocellular Carcinoma

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Background and Aims: Hepatocellular carcinoma (HCC) is a lethal disease due to late diagnosis and a lack of effective screening methods. The Hepatitis B virus (HBV) is the primary etiological factor of HCC in Turkey. miRNA plays an important role in carcinogenesis and may serve as a non-invasive biomarker. In the literature, miR-4510 and miR-146b-5p expression levels were found to be associated with HCC however their associations with HBV related HCC yet to be explored. This study aimed to assess the predictive and prognostic value of expression levels of serum miR-4510 and miR-146b-5p in the HCC with HBV etiology and performed bioinformatics analyses based on the miRNA expression profile.

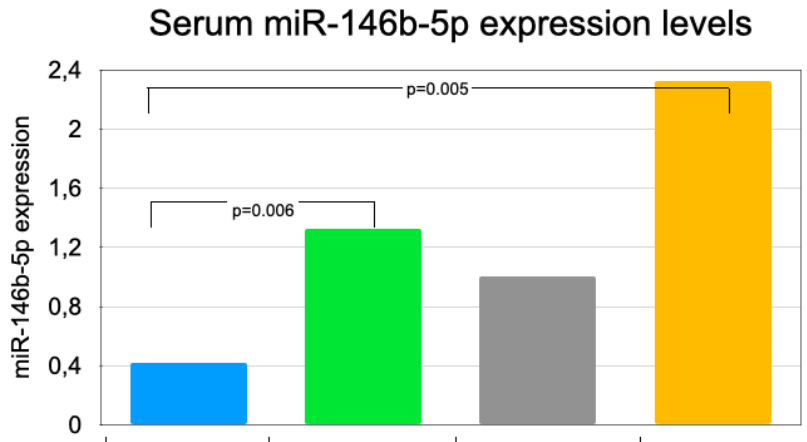
Method: This cross-sectional study used serum from 16 patients with Chronic Hepatitis B (CHB), 15 HBV-cirrhosis, 15 HBV-HCC, and 16 control subjects. The total RNA was isolated from serum and the expression of miR-4510 and miR-146b-5p were measured by qRT-PCR calculated using $2^{-\Delta\Delta Ct}$ methods. MIENTURNET and Network Analyst were used to predict miRNA-target interactions and visualization of functional enrichment analysis.

Results: There was a significant difference between study groups for miR-146b-5p (p=0.009) (Figure1.). MiR-146b-5p expression was significantly reduced in only HBV-HCC group and HBV-Cirrhosis group than in controls (p=0.005 and p=0.006 respectively) (Figure 1). No significant association was found between the miRNA-146b-5p and clinicopathological parameters (p>0.05). MiR-4510 expression was not detected in all patient groups but only in 3 individuals in the control. There was not found to be any associations between HCC prognosis and miRNAs expression levels.

Conclusion: We concluded that serum miR-146b-5p levels can be used as non-invasive diagnostic biomarkers for HCC but not as prognostic markers. According to our preliminary data, serum miR-4510 expression hasn't diagnostic value for HCC. Taken together, our findings shed light on potential biomarkers for the diagnosis of HBV-HCC in terms of selected miRNAs. MAPK signaling pathway and Pathway in cancer were statistically significant pathway in KEGG. This work has been supported by Marmara University Scientific Research Projects Coordination Unit under grant number SAG-C-DRP-120619-0229.

Keywords: HCC, HBV, miRNA, Biomarker, Cancer

Figure: Relative expression levels of miR-146b-5p in serum.



	HBV-HCC	HBV-Cirrhosis	CHB	Control
Mean (Range)	0.415 (0.13-1.03)	1.326 (0.26-4.20)	1.007 (0.17-3.61)	2.3256 (0.23-16.62)

The relative expression levels of serum miR-146b-5p in 4 groups. The horizontal lines indicate the mean. P values were generated in a Mann-Whitney U test; $P < 0.05$ was considered to indicate statistical significance. There was a significant difference between study groups for miR-146b-5p (p=0.009)

P08-14
Inhibited surface Na+ taurocholate cotransporting polypeptide expressions in hepatocellular carcinoma: A defense mechanism?

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Background and Aims: Na+ taurocholate co-transporting polypeptide (NTCP) expressed exclusively at the basolateral membrane of hepatocytes in the liver and mediates the uptake of glycine/taurine conjugated bile acids (BAs) from the portal vein. Studies showed NTCP involvement in cholestasis, however, NTCP role in Hepatocellular Carcinoma (HCC) was not studied previously. We aim to evaluate NTCP expressions and BAs trafficking in HCC.

Method: Hep3B cells (human hepatocellular carcinoma) were assessed for NTCP expressions through flow cytometry and western blot analysis. Expressions of CYP27A1 (BAs synthesis enzyme) were asses through western blot. Hep3B were treated with BAs substrate; Taurocholic acid (TCA) and with NTCP antagonist; Obeticholic acid (OCA) at concentrations 10 mM and 50 mM. Bile salt export pump (BSEP) were also evaluated. Flow cytometry, western blot and confocal microscopy were also used to evaluate HCC expressions of FXR and BSEP. Apoptosis/necrosis were also evaluated for apoptosis (Annexin-V /PI).

Results: Flow cytometry analysis indicated a diminished expressions of NTCP on HCC and was associated with no BAs trafficking /entry following treatments with TCA. In parallel, quantitation of western blot data showed NTCP immunoblot bands indicating an intracellular NTCP expressions. Also, data showed protein expressions of CYP27A1 and BESP indicating an active mechanism of intracellular synthesis of BAs and BAs exporter, respectively. Compared to normal hepatocytes, intracellular expressions of CYP27A1 in the HCC were 2-fold higher and BAs concentrations were 4-fold elevated ($P<0.05$). These results were correlated with increased FXR in HCC and as a result inhibited intracellular NTCP ($P<0.01$). OCA-treated HCC significantly activated FXR expressions and negatively regulated intracellular expressions of NTCP. Moreover, OCA while decreased apoptosis of HCC from 60±7% to 39±3.1% (annexin-V+/PI-) it shifted the cells to late apoptosis and necrosis from 26±4 to 50±6.6 % (annexin-V+/PI+). αFP levels were reduced from 250±27 ng/ml in untreated HCC to 89±11 ng/ml in the OCA-treated cells ($P<0.05$). There results were linearly correlated with the increase concentration of 50 mM OCA.

Conclusion: Our data indicated an inhibited surface expressions of NTCP which could indicate protective effects against insults of BAs. In turn, high synthesis of BAs (most probably because of high proliferative cells) increased FXR and lower intracellular NTCP expressions which in part indicated NTCP translocation dysfunction and may explain escape/defence of the cells and direct them to late apoptosis. Modulatory therapies targeting FXR could be a potential approach to prevent complications to liver cancer.

P08-18
Immunomonitoring of hepatocellular carcinoma patients undergoing liver transplantation after treatment with Bevacizumab and Atezolizumab

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Background and Aims: In hepatocellular carcinoma (HCC) patients undergoing tumor downstaging by anti-angiogenic therapy (AAT) + immune checkpoint inhibitors (ICIs), liver transplantation (LT) represents a potential curative option. In other cancers, tumor regression is due to the ICI-mediated activation of memory and effector T cells and AAT mediated down-modulation of myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg). In the LT setting immunosuppressant therapy and the prolonged ICI washout applied for minimizing graft rejection, could blunt ICI-promoted immune surveillance. In this study we performed a comprehensive blood immune profile of HCC patients undergoing LT after atezo+bev, to monitor tumor immunity in relation to treatment and clinical outcomes.

Method: 3 HCC patients downstaged with atezo+bev and receiving LT were studied during pre-LT washout (T0 and T1) and at different post-LT intervals (T2-T4, Figure 1).

Results: According to flow cytometry profiling, pre-LT ICI washout was associated with a rapid drop of effector/memory (EM) and terminally-differentiated effector memory (TEMRA) T cell responses with respect to the levels achieved during ICI-AAT treatment. Concomitantly, immunosuppressive pro-tumoral cell subsets, including monocytic MDSC (M-MDSC), granulocytic MDSC (PMN-MDSC) and Treg, remarkably increased (Figure 1). After LT, all T cell subsets were further reduced, albeit with different kinetics. In contrast, M-MDSC and Tregs progressively raised in a patient-specific manner, while PMN-MDSC remained at low levels (Figure 1).

Conclusion: These preliminary results suggest that ICI-AAT pre-LT washout is associated with a rapid loss of the anti-tumor immunological benefit achieved in peripheral blood by atezo+bev. To what extent the balance of pre-LT ICI washout and post-LT immunosuppression may modify the atezo+bev anti-tumor effect needs to be assessed at longer intervals together with the relative weight of each component.

Figure:

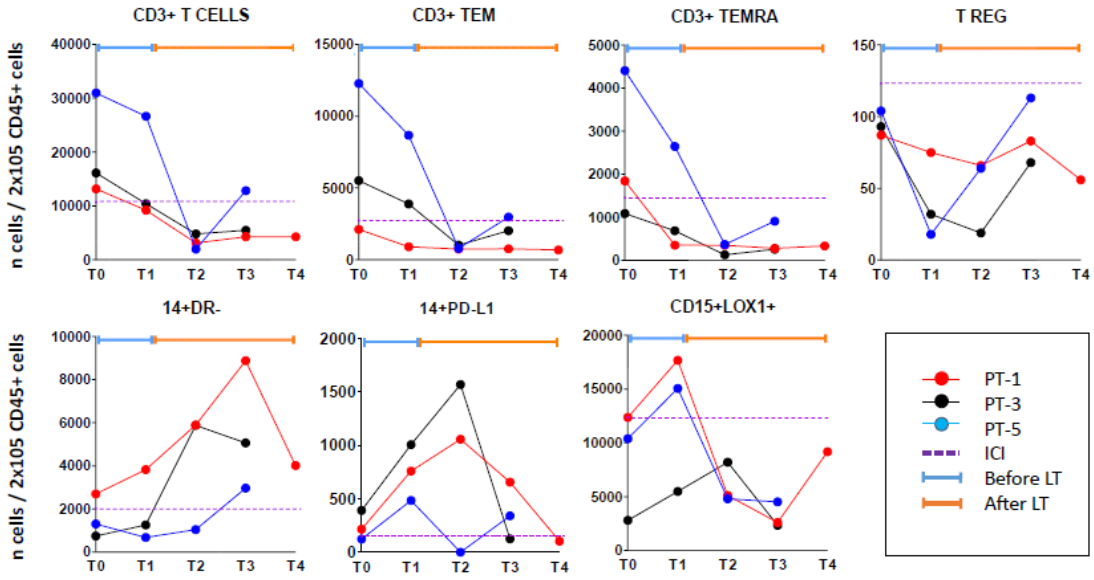

Fig. 1. Preliminary data obtained from flow cytometry analysis of HCC patients undergoing LT after ICI+AAT. High resolution flow cytometry performed at different time points before and after LT. T0 and T1, during ICI+AAT washout (blue bracket); T2-T4, at day 3, 7 and 14, respectively, after LT (orange bracket). Data were analysed by Kaluza Software.

P09-02-YI
KLF5 upregulation is a common event in cholangiocarcinoma, acting as an oncogene and constituting a bad prognostic factor

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Background and Aims: Cholangiocarcinoma (CCA) comprises a heterogeneous group of malignant tumors with poor prognosis. Krüppel-like factors (KLF) are a family of transcription factors involved in large variety of biological processes, including organogenesis, differentiation and cellular homeostasis. Here, we investigated the role of KLF5 in cholangiocarcinogenesis and evaluated the therapeutic potential of its inhibition during CCA tumorigenesis.

Method: *KLF5* expression was determined in human CCA tissues [Copenhagen (n=210), TCGA (n=36), Job (n=78), TIGER-LC (n=90) and San Sebastian cohorts (n=12)] and cell lines. *KLF5*$^{-/-}$ CCA cells were generated by CRISPR/Cas9. Proteomic analyses were carried out by mass spectrometry and the functional effects of KLF5 genetic ablation or chemical inhibition with ML264 were evaluated *in vitro* and *in vivo*.

Results: KLF5 expression was upregulated in human CCA tissues from 5 different patient cohorts compared to surrounding normal liver tissue. High KLF5 levels correlated with lymph node invasion and worse overall survival. In vitro, KLF5 protein and mRNA levels were found upregulated in human CCA cells compared to normal human cholangiocytes. Proteomic analysis of KLF5-/- CCA cells revealed that most of the altered pathways are related with the modulation of cell cycle, proliferation, survival and migration. In agreement, KLF5-/- CCA cells displayed decreased cell proliferation, colony formation and migration while promoting cell cycle arrest at G1/S and apoptosis in vitro, when compared with CCA control cells. Instead, no signs of tumor development were evident after subcutaneous or orthotopic injection in a xenograft animal model of CCA. Likewise, pharmacological inhibition of KLF5 with ML264 hampered CCA cells proliferation and migration in vitro and blocked tumor growth in vivo in distinct animal models. Lastly, both genetic and pharmacological inhibition of KLF5 sensitized CCA cells to chemotherapy-induced apoptosis in vitro, and the combination of the standard of care chemotherapy (gemcitabine + cisplatin) and ML264 completely halted CCA tumor growth in mice.

Conclusion: Increased KLF5 is a general event in CCA, contributing to cancer progression by promoting cell survival and proliferation, as well as, chemoresistance. KLF5 inhibition with ML264 may represent a potential therapeutic strategy for CCA.

P09-04-YI
Dissecting the tumor heterogeneity and tumor microenvironment in intrahepatic cholangiocarcinoma using spatial transcriptomics

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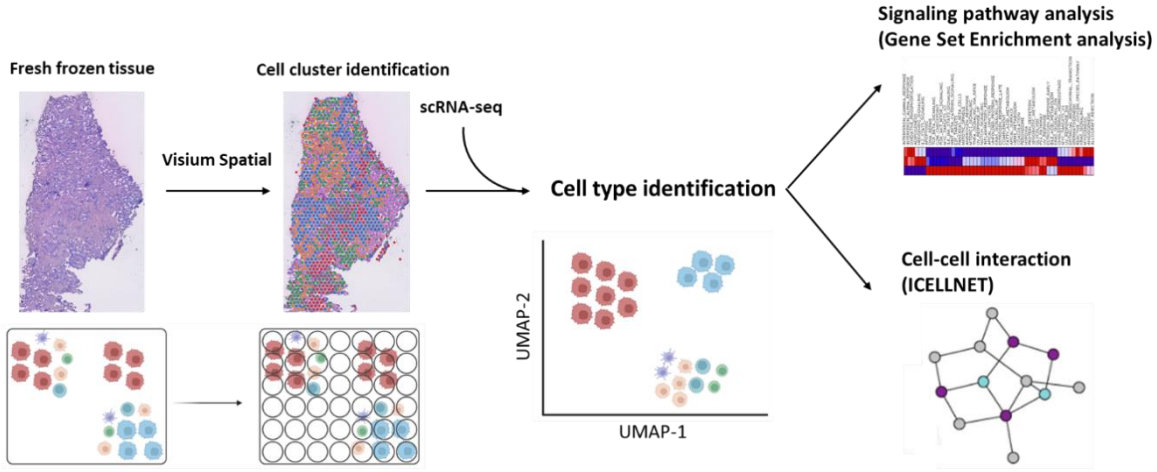
Background and Aims: Intrahepatic cholangiocarcinoma (iCCA) is the second most common primary liver malignancy after hepatocellular carcinoma (HCC) with limited therapeutic options. Immunotherapy (IO) has revolutionized cancer therapy during the last decade, offering durable responses with an acceptable safety profile. In biliary malignancies, addition of the immune checkpoint inhibitor to Gemcitabine/Cisplatin has recently become the standard of care in first line treatment, however, the overall survival benefit is moderate in an all-comer population. To facilitate patient stratification, and to explore new strategies, it will be critical to characterize and better understand the interplay between tumor cells and the immune microenvironment.

Method: We combined spatial transcriptomics (ST) with published single-cell RNA sequencing (scRNA-seq) of iCCA patients to identify the subpopulations of tumor cells and spatial structures in the tumor microenvironment. We also inferred cell-to-cell relationships from high throughput ligand-receptor interaction measurements within tissue sections.

Results: High-resolution spatial transcriptomic analysis reveals subpopulations of tumor cells that display distinct patterns of KRAS-, Myc-, TGFß- and Wnt/beta-catenin signaling pathway signatures. We also observed a higher frequency of a tumor immune barrier structure in stroma rich tumor, while stroma poor tumor cells are more frequently infiltrated with macrophages/T-cells. In stroma rich tumor, tumor cells express IGF1R while surrounding fibroblasts express IGF1, suggesting a potential activation of IGF1 pathway which is known to promote tumor growth and survival. In both, stroma rich and poor tumor, tumor cells express vascular endothelial growth factor VEGFA, which can stimulate endothelial cells through VEGF receptor, FLT1, suggesting a pro-angiogenic shift in the state of the tumor-associated endothelial subpopulation. In addition we identified macrophage subpopulations with a high M2-gene signature. Interestingly, we detected CXCL6+ tumor cells, suggesting a tumor-associated microbiota. We are currently determining the identity and *in situ* location of intratumoral microbial communities within patient tissues.

Conclusion: Our data suggest that an immune suppressive tumor microenvironment associates with suppressive myeloid populations, in addition to high stromal angiogenic activity. Our work thus provides a highly detailed and comprehensive analysis of the iCCA tumor microenvironment and an exploratory analysis of tumor-stromal cell interactions.

Figure: Workflow description (partly created with BioRender.com)



P09-06
The in ovo and ex ovo chick chorioallantoic membrane assay as an efficient xenograft model to study anticancer drugs activity in hepatocellular carcinoma

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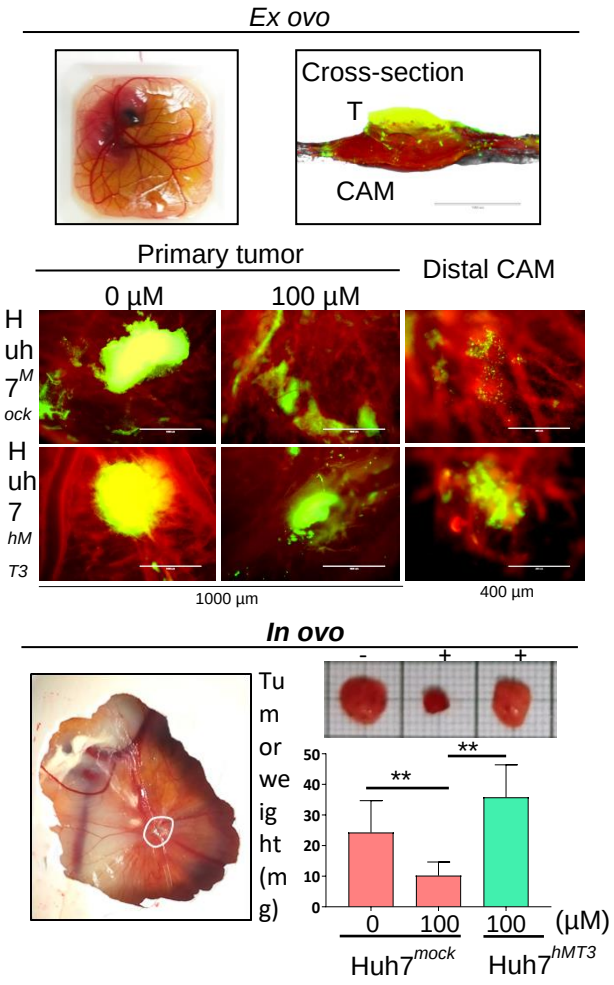
Background and Aims: The chick chorioallantoic membrane (CAM) is naturally immunodeficient and highly vascularized, making it an ideal system for tumor implantation. Sorafenib is a widely used first-line standard systemic agent for therapy of advanced hepatocellular carcinoma (HCC). It has been suggested that elevated levels of some metallothionein (MTs) isoforms could mediate protection against apoptosis and promote cell proliferation, thus encouraging chemoresistance. Thus, we decided to develop *in vivo* preclinical models to study the activity of sorafenib in human MT3 overexpressing HCC cells Huh7 by *in ovo* and *ex ovo* CAM assay.

Method: Fertilized chicken eggs were obtained from local farm. The eggs were incubated with intermittent rotation. For *ex ovo* CAM assay, the eggshells were cracked and embryos were transferred into small, sterile plastic bowls. Then, bowls were covered with a square Petri dish and placed in incubator until day 10, when embryos were xenografted on several places. Before xenografting, $Huh7^{mock}$ and $Huh7^{MT3}$ cells were pre-labeled with CellTracker Green and implanted on the CAM at an initial seeding density of 5×10^4. After 3 days of incubation, 100 µM sorafenib was added to each microtumor and *ex ovo* cultures were further incubated for 24 h. For subsequent fluorescent photos, EVOS FL Auto Cell Imaging System was used. For *in ovo* CAM assay, after 10 days, a square window was drilled into eggshell. Then, eggs were inoculated with 1×10^6 $Huh7^{mock}$ and $Huh7^{MT3}$ cells grafted directly on the CAM, followed by incubation (6 days). Then, 100 µM sorafenib was added topically on the upper CAM directly to the developed microtumors. After 24 h, CAM containing tumors, and organs were harvested, weighted and histology process.

Results: After induction HCC xenografts on CAM, highly vascularized HCC tumors, formed from densely populated HCC cells were developed. We also confirmed that the transient up-regulation of MT3 resulted in high proliferation activity of $Huh7^{MT3}$ tumor. It is worth to note that follow-up experiments revealed a significant chemoresistance to sorafenib due to MT3 up-regulation, while sorafenib efficiently decreased weight/volume of $Huh7^{mock}$ tumor. Interestingly, we also found out that although sorafenib exhibited only a slight inhibitory effect on metastatic spread of $Huh7^{MT3}$ cells.

Conclusion: We show that the applicability of CAM assay can be simply extended from its primary purpose to study carcinogenesis to the field of screening of anticancer activity of various types of drugs useful for HCC therapy. Besides, we provide clear evidence that up-regulation of MT3 results in increased metastatic spread from the primary tumors and also enhanced chemoresistance phenotype of examined HCC cells.

Figure:


Ex ovo
Cross-section
T
CAM
Primary tumor
Distal CAM
0 µM
100 µM
Huh7mock
Huh7hMT3
1000 µm
400 µm
In ovo
Tumor weight (mg)
(µM)
Huh7mock
Huh7hMT3

P09-08-YI
Oncogenic role of the Hepatitis B Virus surface antigen during hepatocarcinogenesis

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Background and Aims: Hepatitis B virus (HBV) infection is the leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). The accumulation of HBV surface antigen (HBsAg) induces inflammatory response together with direct activation of oncogenes leading to HCC. This study aim is to investigate the dynamics of various oncogenes during hepatocarcinogenesis related to HBV infection in a HBV-transgenic mouse model.

Method: A total of 92 tissue samples from HBV-transgenic mouse model C57BL/6J-TG(ALB1HBV)44BRI/J (HBV-TG) and its wildtype counterpart (C57BL/6J) (WT) were analyzed. Liver samples were collected representing the different stages of injuries due to the presence of HBsAg (3 months: inflammation, 6 months: early hepatic damage, 9 months: dysplasia, and >12 months: neoplasia). Serum transaminases and lactate dehydrogenase (LDH) levels were measured to confirm hepatic injury. *In silico* analysis of protein-protein interaction (PPI) networks from published HCC classifications identified the oncogene candidates of this study. Gene expressions analysis was performed by RTqPCR to investigate the differences of mRNA expression across age groups and between strains. Statistical analysis was calculated using GraphPrism software.

Results: *In silico* analysis identified eleven candidate oncogenes in this study: c-Fos, Fgr, Mdm-2, Zbtb16/PLZF, Jun/AP-1, Cblb, Dcun1d2/DCNL2, Eps15, Src/pp60c-src, Aurka/AIRK1, and Yap1/Yap65.There were upregulated expressions of Aurka (Aurora Kinase A) and Src (Rous sarcoma oncogene) for about 3-fold higher and 2- to 5-fold higher, respectively, in HBV-TG compared to WT ($p<0.0001$). Paired analysis within similar age groups confirmed the observed differences. Interestingly, the expression level of Src in HBV-TG liver tissues sequentially increased along with the progression of liver. The increase of Aurka was already noted to be significantly high at 3 months in HBV-TG. This might be related to the presence and accumulation of HBsAg in the liver. In parallel, the increase of alanine transaminase (ALT) and aspartate transaminase (AST) were significantly increased along with hepatic injury in HBV-TG mice.

Conclusion: The role of oncogenes such as Aurka and Src with the presence and accumulation of HBsAg may contribute to the initiation of hepatocarcinogenesis.

P09-10
Novel approaches to improve immunotherapy for NASH-induced HCC

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Background and Aims: Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer. In Europe and North America, 15%-20% of HCC cases attribute to NASH, but this percentage is expected to increase in the near future.

Recent study found that immune checkpoint inhibitors in combination with anti-angiogenic drugs exert a potent anti-tumor effect toward advanced HCC, probably through breaking immunosuppressive TME3. However, in the pre-clinical model of NASH-HCC, in which mice were fed a choline-deficient high fat diet (CD-HFD) or Western diet (WD), Pfister. D *et al*, reported that anti-PD-1 treatment could not reduce tumor burden, although the CD8+PD1+ T cells were increased within the tumor after treatment. A meta-analysis of three randomized phase III clinical trials that tested inhibitors of PDL1 (programmed death-ligand 1) or PD1 in more than 1,600 patients with advanced HCC revealed that immune therapy did not improve survival in patients with non-viral HCC. These data suggest that NASH/NAFL in the context of HCC potentially influence the effectiveness of an immunotherapy response.

Thus, further studies are warranted to understand the cross-talk and interplay among immune cells and cancer cells, which facilitate to develop better immunotherapy against NASH-NAFL induced HCC.
We aim to develop an improved immunotherapy of HCC in a preclinical model of HCC with underlying NASH by novel combinatorial treatments. Since NASH is a metabolic disease, we hypothesis that combining distinct anti-metabolic-regulating reagents with immunotherapy would be beneficial for the NASH-related HCC.

Method: We use the CD-HFD- or WD-induced HCC model, because these are the best models to mimic the chronic development of NASH and spontaneous development NASH-HCC in human patients. Metabolic-regulating reagents, which have been reported in NASH or HCC treatment, are taken as the candidates. The NASH-HCC tumor metabolites are screened by gas chromatography–mass spectrometry (GC-MS).

Results: We found that combining metabolic-regulating reagents with immunotherapy, compared with mono-immunotherapy, would efficiently reduce the growth of tumors in NASH-HCC moues models. Interestingly, combining metabolic-regulating reagents with immunotherapy could significantly reduce the body weight, which mainly attribute to the loss of fat tissue. By GC-MS metabolites screening, we found that metabolic-regulating reagents could change the metabolism of tumors, release the metabolic stress of NASH-HCC microenvironment and revert the T-cell phenotypes.

Conclusion: Combining metabolic-regulating reagents with immunotherapy could be a novel therapeutic strategy for NASH-related HCC.

P09-12
The role of thyroid hormone signalling in liver carcinogenesis: a proof of knowledge

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Background and Aims: Emerging reports suggest a relationship between thyroid hormones (TH) signalling pathways and liver diseases, including hepatocellular carcinoma. Deiodinases are enzymes involved in serum and hepatic regulation of TH. Although it has been proven that the expression of deiodinases type 1 (D1) and 3 (D3) changes during induced liver injury, their role is still poorly understood in hepatocarcinogenesis. We aimed to evaluate the role of deiodinases and their regulation in liver carcinogenesis investigating whether and how the TH homeostasis impacts on HCC phenotype and patients' outcome.

Method: This is a proof of knowledge introductory to an ongoing monocentric prospective case-control study. We enrolled 19 patients underwent liver surgery for HCC (10 cases) or for other non-neoplastic liver diseases in non-cirrhotic context (9 controls). We evaluated genes and protein expression of the main TH metabolism factors (D1, Monocarboxylate transporter 8 -MCT-8-, Thyroid receptors alpha and beta and Kruppel-like factor 9 -KLF9-), with RT-PCR and Western blot analysis in HCC, cirrhotic liver and healthy liver samples.

Results: RT-PCR analysis showed a progressive statistically significant decrease of D1 (p 0.004), MCT-8 (p 0.001) and TRα (p 0.02) mRNA expression from healthy liver to HCC. The expression of KLF9, involved in cell differentiation and proliferation, decreased accordingly (p 0.03). Western Blot analysis showed a decreased expression of D1 protein in all cirrhotic samples (p 0.01), while D3 increased in 50% of HCC (p 0.02)**.** Among HCC patients, D3 expression was associated with more severe liver stiffness (32 kPa, IQR 23.47-35.5, p 0.002) and high BMI (p 0.004). A statistically significant overall survival (OS) difference between D3 positive and D3 negative HCC patients was observed (log rank p 0.003), with a median OS of 17.9 (IQR 15.5-18.7) months for D3 positive vs 41.3 (35.1-43.8) months in D3 negative **(Fig.1).** Furthermore, a shorter Progression Free Survival and an increased recurrence rate was observed in D3 positive patients, even if not statistically significant.

Conclusion: These preliminary data showed that D3 expression could define a more severe phenotype of HCC and it could be used in clinical practice as negative prognostic biomarker of patients' outcome. Our exploratory findings need to be applied to a larger sample size to be confirmed.

Figure:

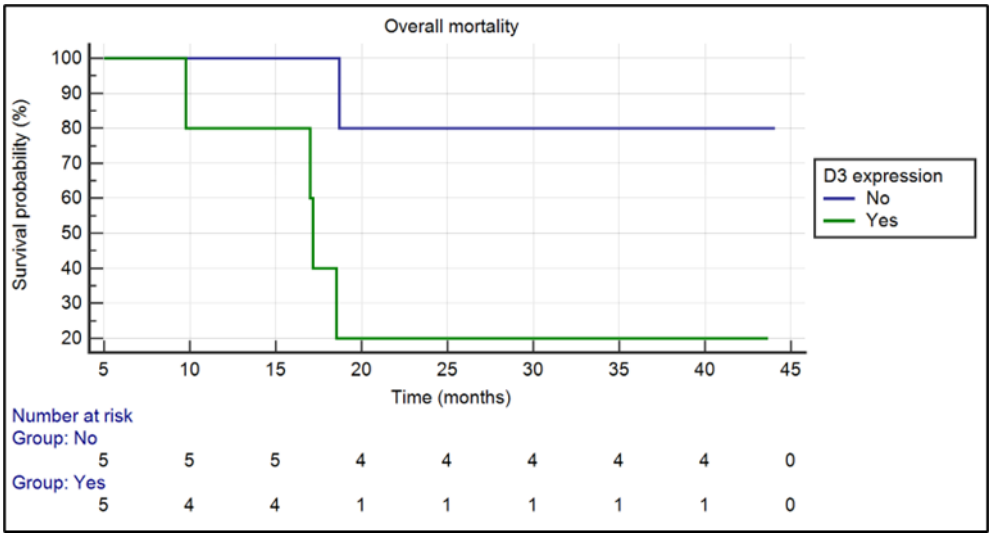

Figure 1. Kaplan-Meyer survival curve in HCC patients D3+ and D3-

P09-14
Effects of Rifaximin on Epigenetic and Autophagy Markers in an Experimental Model of Hepatocellular Carcinoma Secondary to Non-alcoholic Fatty Liver Disease

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Background and Aims: The burden of hepatocellular carcinoma (HCC) associated with non-alcoholic fatty liver disease (NAFLD) is increasing. Preventive strategies are needed to counteract this trend. Thus, we investigated the effects of rifaximin (RIF) on epigenetic and autophagy markers in an experimental model of HCC secondary to NAFLD.

Method: Adult Sprague Dawley rats were randomly assigned (8 animals each) and treated from 5 to 16 weeks with gavage as follows: control [standard diet; water free of diethylnitrosamine (DEN) plus gavage with vehicle (Veh)], HCC [high-fat diet with choline deficiency (HFCD), DEN in drinking water and gavage with Veh], and RIF [HFCD, DEN in drinking water and gavage with RIF (50 mg/kg/day).

Results: Hepatic expression of matrix metalloproteinases (*Mmp*)-9 was higher in the HCC-group in relation to the control, inversely observed for *p62*/sequestosome-1 (*p62/Sqtm1)* ($p<0.05$). There was a significant decrease in autophagy-related-factor-LC3A/B *((Map1l3b)*, beclin (*Becn*)-1 and enhancer of zeste homolog (*Ezh*)-2 in the HCC and RIF-groups compared to the control ($p<0.05$). Coactivator-associated arginine methyltransferase (*Carm)-1* was lower in the HCC compared to the RIF-group ($p<0.05$). There was no difference between groups for tubulin alpha (*Tuba)-1c,* aldolase (*Aldo)-b*, alpha-fetoprotein (*Afp),* and *Mmp2* ($p>0.05$). miR-122 expression was significantly higher in the HCC-group compared to the control ($p<0.05$). miR-34a was significantly higher in the RIF-group compared to the control ($p<0.05$). miR-26b expression was significantly lower in the HCC-group compared to RIF-group, the inverse was observed for miR-224 ($p<0.05$). There was no difference between groups for expression of miR-33a, miR-143, miR-155, miR-375 and miR-21 ($p>0.05$). All animals in the HCC and RIF groups developed NAFLD, however three animals in the RIF-group did not develop HCC. There was a significant positive correlation between miR-122 and miR-34a in relation to the autophagy markers *Becn1, p62/Sqtm1* and *Map1l3b*. There was a significant positive correlation between Becn1, p62/Sqtm1 and Map1l3b and there was no correlation between *Ezh2* and *Carm-1.*

Conclusion: We demonstrated a beneficial effect of the treatment with RIF in rats with NAFLD-HCC in relation to the epigenetic markers evaluated, suggesting it could be useful to prevent or delay carcinogenesis.

P09-16
Liposomal doxorubicin targeted with atezolizumab as a novel strategy to cure HCC: preclinical in vitro and in vivo studies

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Background and Aims: HCC is a major cause of cancer-related deaths, although novel pharmacological regimes, based on immunotherapy, have been approved. This study aims at assessing the efficacy of a novel liposomal formulation loaded with doxorubicin (DXR), targeted with the Fab' of the PD-L1 checkpoint inhibitor atezolizumab, for the selective delivery of the cytotoxic agent to cancer and exhausted immune cells.

Method: In this study, two DXR-loaded liposomal formulations were tested both in vitro and in vivo, i.e., untargeted Stealth Liposomes (SL) and Stealth ImmunoLiposomes (SIL) targeted with atezolizumab. Cytotoxic effect was assessed on human HepG2 and murine Hepa1-6 cells by the ATP assay. DXR internalization was assessed by live confocal microscopy. Their effect on macrophage polarization was assessed on THP1-derived macrophages cocultured with HepG2. A HepG2-based spheroid model was set up to evaluate the effect of SIL and SL on sphericity index (SI) and cell viability by CellTiter-Glo® 3D Cell Viability test. In vivo efficacy was assessed in a mouse syngeneic model of HCC, obtained by injecting Hepa1-6 cells in C57BL/6J immunocompetent mice. They were treated with SIL or SL (10 mg/Kg, 1EV/week for 4 weeks) when tumors reached a volume of 100 mm^2. Tumor volumes were measured by an electronic caliper and collected at sacrifice to evaluate infiltrated macrophages by IHC.

Results: Atezolizumab targeting increased the *in vitro* internalization and cytotoxic effect of liposomal DXR in HepG2 and Hepa1-6 cells, since SIL had IC50 values significantly lower than those of SL ($p<0.05$). SIL-treatment significantly increased the polarization of M0 macrophages toward the anti-tumoral M1 phenotype with respect to control and SL-treated cells ($p<0.01$) and effectively reduced spheroid viability (IC50=1.122 mM) without affecting SI. Both formulations decreased tumor growth *in vivo* (tumor volumes measured at sacrifice were significantly lower in treated mice compared to controls, $p<0.05$). The number of pro-tumoral macrophages was significantly lower in treated mice, particularly in those treated with SIL.

Conclusion: PD-L1-targeted liposomal DXR reduces cancer cell growth in HCC preclinical models and prompts macrophage polarization towards an antitumoral phenotype. The active targeting of DXR to tumor cells and exhausted immune cells may pave the route to new pharmacological treatments of HCC, able to reduce off-target toxicity and enhance anticancer activity.

P10-02
Six-transmembrane epithelial antigen of prostate 4 Expression in Hepatocellular Carcinoma: Correlation with Tumor Progression and Immune Response

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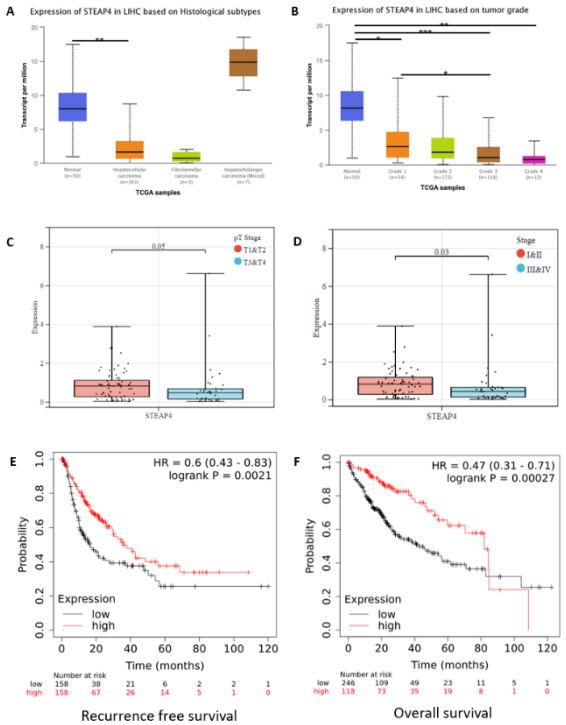
Background and Aims: The six-transmembrane epithelial antigen of prostate 4 (STEAP4) has been shown to promote tumor progression associated with its roles in inflammatory reactions, oxidative stress, and metabolism. However, STEAP4 has rarely been studied in hepatocellular carcinoma (HCC). We explored STEAP4 expression associated with tumor prognosis to understand its role in tumor biology in HCC.

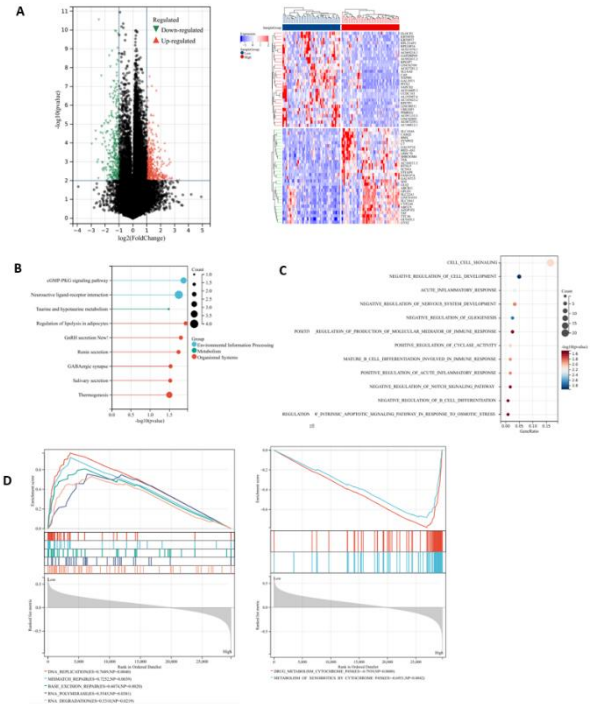
Method: STEAP4 mRNA and protein expressions were primarily analyzed based on The Cancer Genome Atlas database using bioinformatics tools to understand the expression pattern, molecular mechanism, prognostic impact, and association with immune cell infiltration. We further investigated the association between STEAP4 protein expression and clinicopathological parameters and their predictive value in patients with HCC using immunohistochemical staining (IHC) of tissue microarrays.

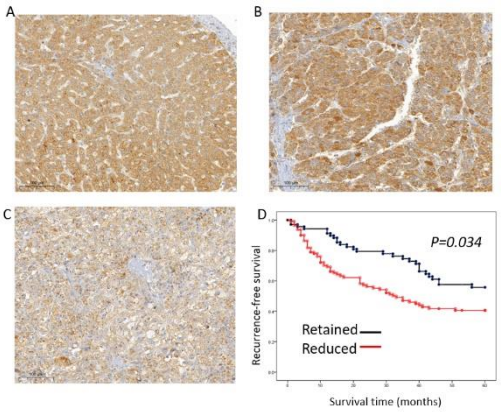
Results: The expression of STEAP4 mRNA and protein was found to be significantly lower in HCC tissues than in normal liver tissues. HCC with advanced stages, poor relapse-free survival (RFS), and overall survival were associated with reduced expression of STEAP4. Furthermore, reduced STEAP4 expression was a significant predictor of worse recurrence-free survival in univariate and multivariate analyses in IHC cohort. GO, KEGG, and GSEA analyses indicate that STEAP4 is related to numerous biological processes and pathways, such as drug metabolism, DNA replication, RNA metabolism and immunoresponse. In terms of the immune system, the decreased level of STEAP4 was correlated with the immunosuppressive microenvironment.

Conclusion: Our data indicated that reduced STEAP4 expression was significantly associated with tumor aggressiveness and poor prognosis possibly due to its link with various biological processes and triggering immune evasion of HCC. Therefore, STEAP4 expression may serve as a potential prognostic biomarker for cancer progression and cancer immunity, as well as a potential therapeutic target in HCC.

Figure:


A
Expression of STEAP4 in LIHC based on Histological subtypes
B
Expression of STEAP4 in LIHC based on tumor grade
C
D
E
HR = 0.6 (0.43 - 0.83)
logrank P = 0.0021
Recurrence free survival
F
HR = 0.47 (0.31 - 0.71)
logrank P = 0.00027
Overall survival


A
B
C
D


A
B
C
D
P=0.034
Retained
Reduced
Recurrence-free survival
Survival time (months)

P10-04-YI
An innovative human-derived Cholangiocarcinoma-on-chip as a platform for targeted therapy

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Background and Aims: Cholangiocarcinoma (CCA) is a deadly cancer of the biliary epithelium characterized by poor prognosis and limited therapeutic options. This scenario highlights the imperative need for elucidating CCA pathophysiological mechanisms to develop new therapeutic strategies. In the last years, strong efforts have been focused on the Organ-On-Chip (OoC) as a promising tool able to potentially recapitulate an *in vivo*-like 3D environment, compared to the standard 2D culture systems. Therefore, we aimed to develop an innovative CCA-on-chip device as a platform for drug screening purposes.

Method: Primary CCA cells were isolated from patients surgically resected at the Hepatobiliary Surgery Department, Humanitas Clinical and Research Hospital. The microfluidic device was designed and fabricated at Politecnico di Milano, composed of three microfluidically interconnected channels separated by pillars and with independent accesses.

Results: CCA niche was mimicked by co-culturing CCA cells and cancer-associated fibroblasts (CAFs) in the central channel, embedded in a fibrin/collagen hydrogel, and flanked by an endothelial tubule. The device architectural reconstruction showed the ability of the cells to self-assembly in a physiological-like 3D topology. Moreover, the cells displayed a significant increase in the expression of key phenotypic cell markers, compared to 2D culture system. Diffusion assays assessed the high biocompatibility of this platform at small and large molecules, thus identifying the functional integrity of the endothelial tubule, which acted in a size-selective manner. Subsequently, hydrogel mechanical proprieties showed that deep matrix changes characterized the tumor niche during time in culture, highlighted by an increase in hydrogel stiffness and a reduction of its porosity. Besides, we observed a significant increase in the collagen IV deposition in the device at Day 4 compared to Day 1, only when tumor cells were co-cultured with CAFs. Furthermore, drug exposure analysis using Gemcitabine and Cisplatin, the standard of care for CCA patients, revealed the increased resistance of CCA cells co-cultured with CAFs in the device compared to the 2D monolayer culture and the 3D tumor cell mono-culture.

Conclusion: Our results showed that an in vivo-like CCA microenvironment was developed in a 3D microfluidic device, in which an extensive and bidirectional crosstalk was established between CCA cells and CAFs. This platform could represent a reliable 3D platform to elucidate the biological mechanisms involved in CCA chemoresistance and may provide a biologically relevant tool for patient-specific therapeutic strategies, paving the way toward precision medicine.

Figure:

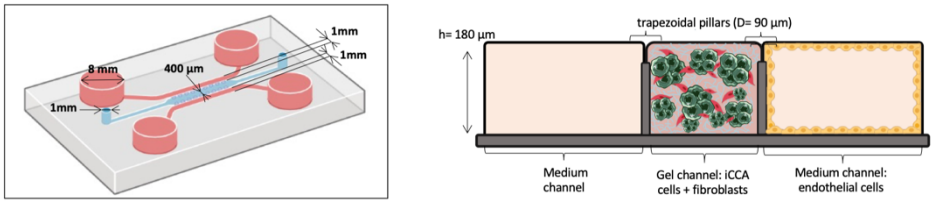

Figure 1: *Schematic representation of the CCA-on-CHIP*

P10-05
Targeting epigenetics in preclinical models of Hepatocellular Carcinoma for translational therapy

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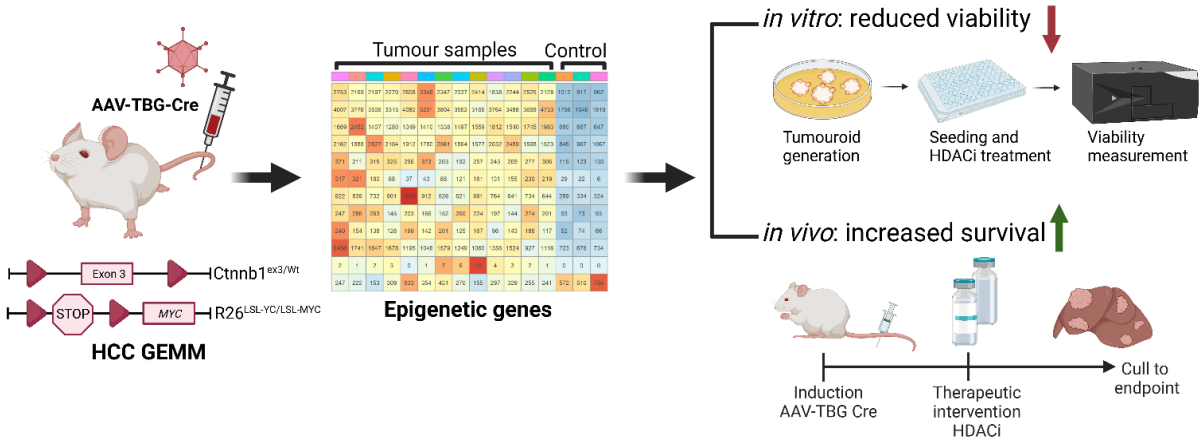
Background and Aims: Hepatocellular carcinoma (HCC), the most common form of primary liver cancer, is the result of a complex interplay between genetic and epigenetic alterations accumulated and selected over time against a background of chronic liver disease. Unlike the genetic drivers of HCC, epigenetic alterations in HCC initiation and progression remain poorly characterized. As sequential biopsies of patients with HCC, from premalignancy to late-stage disease, are rarely performed genetically engineered mouse models (GEMMs) represent a potentially powerful experimental tool to study HCC evolution. Our aim is to investigate epigenetic regulators during tumour evolution and the efficacy of systemic therapeutic interventions targeted at epigenetic regulators. We employ a range of recently developed human-aligned GEMMs to maximise clinical relevancy.

Method: Firstly, we employed a well characterised oncogene driven GEMM, to investigate epigenetic alteration during tumour evolution. AAV-TBG-Cre induction in this mouse model activated *Ctnnb1* and overexpressed *MYC*, resulting in malignant lesions that recapitulate human beta-catenin driven HCC. We used immunohistochemistry staining for specific epigenetic DNA and histone modifications to compare end-stage tumorous tissue vs normal or adjacent. We employed Gene Set Enrichment Analysis (GSEA) in an RNA-seq dataset to explore the epigenetic landscape underlying in our model. Furthermore, we created GEMM-derived, 3D hepatic tumouroids from different mouse models and we proceeded to a high throughput screening using several histone deacetylase inhibitors (HDACi) as *in vitro* treatment. We then used the most effective HDACi, Romidepsin (FK228), in combination with Lenvatinib as a late-stage treatment *in vivo*. Finally, we created novel GEMMs through deletion of an epigenetic regulator associated with HCC, BRCA1 associated protein 1 (*Bap1*) to investigate its tumorigenic potential *in vivo*.

Results: We showed that 3D hepatic tumouroids are responsive to HDACi treatment *in vitro*. These data suggested this response is dependent upon HDAC inhibitor molecular structure and HDAC class selectivity and furthermore influenced by genetic background across a range of different GEMM-derived tumouroids. Romidepsin and Lenvatinib combination treatment *in vivo* led to significant survival benefit and decreased tumour-count in late-stage HCC in the GEMM bearing *Ctnnb1* and *MYC* alterations.

Conclusion: Overall, this preclinical study delineates the crucial role of epigenetic alterations in HCC evolution and expands existing potential treatment options. The close link between *in vivo* and *in vitro* of our adaptable experimental system will allow for rapid validation with significant translational potential.

Figure:


AAV-TBG-Cre
Exon 3
Ctnnb1ex3/Wt
STOP
MYC
R26LSL-YC/LSL-MYC
HCC GEMM
Tumour samples
Control
Epigenetic genes
in vitro: reduced viability
Tumouroid generation
Seeding and HDACi treatment
Viability measurement
in vivo: increased survival
Induction AAV-TBG Cre
Therapeutic intervention HDACi
Cull to endpoint

P10-06-YI
Metabolic rewiring by increased mitochondrial respiration drives immunosuppression in liver cancer

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Background and Aims: Recent evidence supporting the need of a mitochondria-based metabolism for tumor growth prompted us to study the role of MCJ, an endogenous negative regulator of mitochondrial complex I, in the context of hepatocellular carcinoma (HCC). The tumor microenvironment imposes various metabolic regulations to hamper the antitumor immunity of infiltrating immune cells, therefore, modulating the metabolic rewiring may help recover the antitumor immune potential. This work aims to prove increased malignancy in mitochondria-based tumors and to analyze the differential immune response driven by metabolic changes.

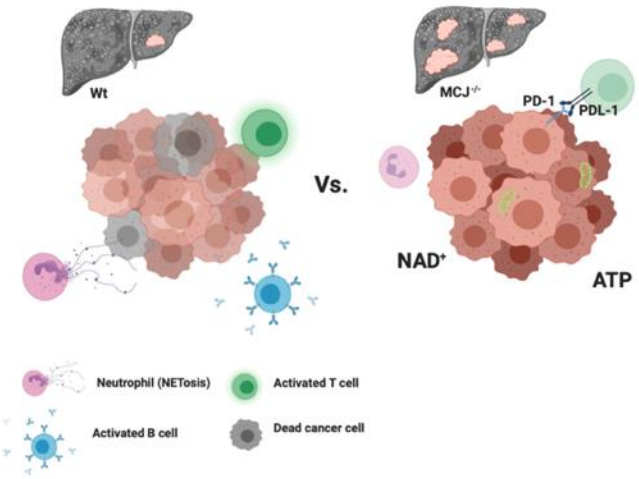
Method: Two different experimental models of HCC were used. Firstly, Wt and whole-body *Mcj*$^{-/-}$ mice were treated with diethylnitrosamine (DEN) for 5,8 or 12 months. Secondly, C57/BL6 mice were injected with MYC-luc;sgp53 plasmid combination and *Mcj* was specifically silenced in the hepatocytes. Tumor progression, liver metabolism, mitochondrial activity and tumor infiltrating cells were assessed in both models.

Results: An in silico approach using UALCAN revealed reduced Mcj expression in patients at stage IV HCC. In vivo, the absence of MCJ increased tumorigenesis and mortality after DEN treatment. A strong oxidative phenotype was confirmed in Mcj$^{-/-}$ tumors, as mitochondrial respiration was significantly higher, with increased intracellular ATP, NAD^+, and NADPH levels. Examination of immune cells infiltrating the tumor showed a reduction in effector T lymphocytes ($CD44^+$ $CD62L^-$) and neutrophils ($GR1^+CD11b^+$) in Mcj$^{-/-}$ mice. Serum and liver cytokine analysis showed a decrease in inflammatory IFNg and TNF in Mcj$^{-/-}$ mice. Significantly increased PDL-1 levels in Mcj$^{-/-}$ mice indicated possible immunosuppression. On the other hand, liver-specific silencing of Mcj enhanced tumorigenesis in the MYC-luc;sgp53 model, along with reduced inflammatory IFNg and TNF and increased PDL-1. Interestingly, 20% of *Mcj*-silenced mice developed brain metastases. Mechanistically, increased expression of ectoenzymes *Cd39* and *Cd73* was found in Mcj$^{-/-}$ mice, along with higher hepatic adenosine levels, which may promote immunosuppression in T cells via adenosine receptor signaling cascades.

Conclusion: Overall, decreased MCJ levels in the liver, which are also seen in advanced HCC patients, promote oxidative respiration and lead to metabolic rewiring that impedes antitumor immune potential

via ATP-ectoenzyme-adenosine signaling and promotes tumorigenesis and even metastasis. Therefore, measurement of MCJ levels along with characterization of glycolytic versus oxidative respiration could help determine the most appropriate treatment, such as blockade of the adenosine axis in combination with immunotherapy.

Figure:



P10-07-YI
T cell and myeloid cell interactions differ in tumour and non-tumour regions in hepatocellular carcinoma

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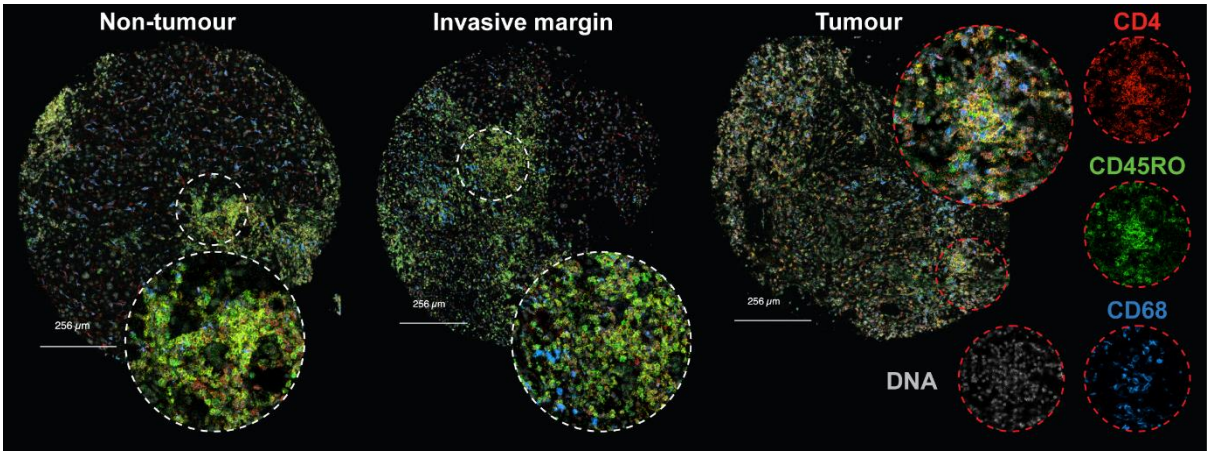
Background and Aims: The tumour microenvironment (TME) is made up of cancer, immune and stromal cells. The specific presence or absence of these cells has been implicated in the pathogenesis of hepatocellular carcinoma (HCC). The TME landscape in HCC has also been associated with clinical and prognostic outcomes. A better understanding of this intricate network is required to enhance the efficacy of current therapeutic options. Our work aims to provide further insight into the HCC TME landscape using imaging mass cytometry (IMC) to map cellular interactions in both a quantitative and spatial context.

Method: Sixteen treatment naïve patients with HCC undergoing curative resection at a single tertiary institution were identified. Two tissue microarrays (TMAs) were created from regions of interest within the resected non-tumour, invasive margin, and tumour tissue. Using IMC, a high dimensional imaging platform that facilitates the quantification of up to forty markers, we developed and optimised an antibody panel consisting of immune, stromal and tumour markers to evaluate in the TMAs. Quantification and spatial analyses were undertaken to interrogate differences between tissue regions. X-shift clustering was done to identify cell subsets. The interaction levels were quantified by calculating the average number of cells within 20 µm. Manual gating was also performed to validate the identification of clusters.

Results: Our high-dimensional panel allowed the identification of 53 subsets of cancer, immune and stromal cells. After quantifying cellular interactions, a sparse partial least-squares discriminant analysis was done. This revealed CD4+ CD45RO+ T cells and CD68+ myeloid cells had a higher level of interaction within tumour compared to non-tumour regions. This was confirmed by manual gating and by inspecting representative images (Figure, blue = CD68+ myeloid cells, yellow = CD4+ CD45RO+ T cells).

Conclusion: Our analyses show differing T cell and myeloid interactions in tumour and non-tumour tissue. Further analysis will provide insight into the contributions of these interactions and their association with relevant clinicopathological parameters.

Figure:


Non-tumour
Invasive margin
Tumour
CD4
CD45RO
CD68
DNA
256 µm
256 µm
256 µm

P10-09-YI
Uncovering epigenetic essentialities of intrahepatic cholangiocarcinoma

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Background and Aims: Patients with advanced intrahepatic cholangiocarcinoma (iCCA) average a survival rate of 12 months on chemotherapy, highlighting an extensive degree of innate and adaptive chemoresistance. A few targeted therapies have been developed for patients with specific genetic mutations (i.e. in FGFR2 or IDH1), but most patients still lack clear therapeutic targets identified by DNA profiling. These tumors often present aberrant transcriptomes, which are maintained across cell divisions thanks to an also altered epigenome. Our aim was to uncover the specific epigenetic regulators that sustain the survival of iCCA using patient-derived tumor models.

Method: Differential expression of epigenetic regulator families (n = 13) was performed using gene set variation analysis (GSVA) on transcriptomes from 357 resected iCCA, 275 matched surrounding livers (SL) and 9 independent normal bile duct (NBD) samples. Pathway overrepresentation analysis was performed using the Enrichr tool. We utilize 3 primary patient-derived iCCA cell models, as well as immortalized cholangiocyte (H69) and hepatocyte (THLE5B) cell lines, to generate a panel of Cas9-expressing stable models and perform a pooled epi-CRISPR inactivation screen, with a custom library of epigenome regulators and other DNA-interacting factors (1386 genes, ~12500 sgRNAs, ~1000 non-targeting control sgRNAs). Essentiality scores were calculated with the MAGeCK pipeline, applying the robust-rank aggregation (RRA) algorithm for reliable identification of epi-CRISPR screen hits.

Results: Our analyses show that 85% (11/13) of epigenetic regulator families are recurrently deregulated in iCCA compared to SL or NBD. Knock-out of epigenetic regulators by pooled CRISPR screening identified diverse pro- and anti-survival phenotypes after gene ablation. 3% (38/1386) of genes negatively impacted tumor cell survival without affecting normal cholangiocytes or hepatocytes, suggesting potential therapeutic windows to modulate these genes. This set of tumor-specific dependencies, enriched on histone modifiers ($p = 0.0063$), is commonly upregulated in iCCA compared to SL ($p < 0.001$ in 4 independent iCCA cohorts, n = 357 patients). Characterization of factors consistently upregulated in iCCA and essential for more than one tumor model showed that, biologically, most of the target genes (4/7) are associated with DNA repair processes (APEX1, PRKDC, MCRS1 and CDK2), indicating a central dependency of iCCA cells on epigenetic factors participating in the maintenance of genome stability.

Conclusion: The survival of iCCA cells is reliant on key epigenetic regulators in a manner that is distinct from the normal liver. Our results support the need to further study the effects of crucial epigenetic factors, particularly regulating DNA repair mechanisms, and their impairment on the morpho-phenotypic level and as therapeutic targets.

P10-12
Expression of MIR-199-5p in chronic viral hepatitis and hepatocellular carcinoma

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Background and Aims: Circulating miRNAs regulate gene expression and are involved in the pathogenesis of chronic viral hepatitis. Modulation of miRNA expression in vitro and in vivo has demonstrated an important role for miRNAs in liver diseases such as viral hepatitis, liver fibrosis, and hepatocellular carcinoma. Non-invasive assessment of liver fibrosis is a promising direction of hepatology and is particularly relevant for detecting, preventing, and treating chronic viral hepatitis. In our study, we focused on miRNA-199a-5p since it is expressed at different levels depending on the type of virus in hepatocytes and has been associated with viral replication and hepatic fibrosis. Mature miRNA-199a plays a critical role in maintaining homeostasis and regulating malignant pathogenesis, according to in-vitro functional studies. Numerous studies have demonstrated that miRNA-199a regulates the activity of normal cells so that they can participate in physiological or pathological processes

Method: In this study, we collected serum samples from 86 patients with viral hepatitis of different etiologies: chronic HCV - 32, chronic HBV - 25 and chronic HDV - 29. Blood serum and liver biopsies were also collected from 32 patients with HCC and 7 liver biopsies from non-affected areas of the liver of these patients. Among the control group were 30 people without HBV, HCV or HDV markers. RNA was isolated using the miScript PCR system (Qiagen, Germany) with C. elegans miR-39 as a normalization control according to the manufacturer's recommendation. We analyzed the relative expression of miR-199a-5p using the 2(-Delta Delta CT) method.

Results: We examined the expression of miRNA-199a-5p in the blood serum of chronic hepatitis B, C, and D patients. All groups had higher levels of this miRNA compared to healthy controls (2(-Delta Delta CT)–20.4, 8.6, 133.3, and 1.2, respectively). MiRNA-199a-5p was most abundant in chronic hepatitis D patients. MiRNA-199a-5p levels in the blood serum of patients with HCC of viral etiology did not differ significantly from control values. At the same time, liver biopsies of these patients had lower expression levels than healthy liver tissue (2(-Delta Delta CT) - 0.3 and 1.6).

Conclusion: Circulating miRNAs play an important role in pathogen-host interactions, and the search for promising miRNA profile indicators in liver fibrosis, liver cirrhosis, and HCC associated with HBV, HCV, and HDV infection will allow their use as novel and non-invasive biomarkers. Monitoring of changes in circulating miRNAs will allow the identification of HBV, HCV, and HDV-infected patients with an increased risk of developing HCC at an early stage of the disease.

P10-13
Poor sorafenib response in hepatocellular carcinoma is mediated by hypoxia-related 14-3-3 scaffolding proteins and induces shift in immune micromilieu

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Background and Aims: Advanced stage of hepatocellular carcinoma (HCC) is frequently accompanied by poor response to the drug treatment or relapse quickly after initial remission. Therefore, identification of molecular drivers of poor response to sorafenib with large focus on novel prognostic markers associated with observed distinct tumor immune landscapes was our ultimate goal.

Method: From a cohort of 91 patients treated with sorafenib, we identified 17 HCC patients with particularly good or bad response. Whole-exome sequencing and integrative RNA sequencing analyses were performed to identify predictive markers of sorafenib resistance. *In vitro* validation of defined targets were performed in a model of sorafenib resistance, followed by subsequent functional and mechanistic validation.

Results: Patients with worst response (n = 7) were characterized by significantly shorter treatment duration and poor overall survival than good responders (n = 10). Molecular analyses revealed that acquisition of drug resistance observed in poor responder group was associated with upregulation of hypoxia-related targets from 14-3-3 scaffolding protein family. WST-1 viability assay displayed that hypoxia contributes to sorafenib resistance. Specific peptide inhibition of this protein family, in combination with sorafenib, showed synergistic effects and efficiently reduced cell proliferation and viability. Dual inhibition consequently reversed sorafenib resistance under both conditions, normoxia and hypoxia, with predominant effects noticed in normoxia. Furthermore, a shift in immune-cell composition with predominant enrichment of M2-immunosuppressive macrophages in worst responders was observed.

Conclusion: Defining the actionable targets of resistance and their subsequent inhibition might greatly help delineate molecular alterations driving drug resistance. In our model, specific peptide inhibition of 14-3-3 scaffolding proteins, when applied in combination with sorafenib, showed a positive correlation in reversing sorafenib resistance. Importantly, synergistic effects of this dual inhibition influenced sorafenib resistance in normoxic and hypoxic microenvironments, but with different potency. This highlights the significance of the tumor microenvironment in modulating the therapy response. Also, greater focus on characterization of the immune micromilieu in different subgroups of patients could be of particular importance to depict treatment resistance and warrants further investigations.

P10-14
Oncostatin M promotes a pro-tumorigenic inflammatory response in NASH-related HCC

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Background and Aims: Oncostatin M (OSM) is a pleiotropic cytokine belonging to the interleukin (IL)-6 family that acts on a large variety of cells. OSM has been proposed to contribute to the progression of chronic liver diseases, hepatocellular carcinoma (HCC) development and metastasis. High levels of OSM were found in cirrhotic patients with different etiology carrying HCC and OSM serum levels are significantly higher in patients carrying non-alcoholic steatohepatitis (NASH)-related HCC, as compared to those whit viral etiologies. Noteworthy, OSM serum levels are significantly higher in patients with intermediate/advanced HCC and correlate with poor survival.
This work discusses the role of OSM in relation to the development of HCC in a NASH background.

Method: We investigated the role of OSM in NASH-related HCC taking advantage of: a) cohort of NASH patients with HCC; b) THP1 macrophage cell lines exposed to human recombinant OSM (hrOSM); c) *Wild type* (wt) mice fed with a control diet (CSAA) or a lipogenic diet (CDAA) for 24 weeks to reproduce the non-alcoholic fatty acid disease (NAFLD)/NASH pathogenic phenotype (CSAA-CDAA protocol); d) *Wild type* (wt) and OSMRβ *knock out* (OSMRβ$^{-/-}$) mice treated with a protocol of NASH-related liver carcinogenesis (DEN/CDAA protocol).

Results: In patients with NASH-related HCC, OSM is expressed in cancer cells in relation to CD68^{+} macrophages infiltrating tumour. In *in vitro* experiments (THP1 cells exposed to hrOSM), we found that OSM is able to promote an M2 pro-tumorigenic phenotype by involving STAT3 and PI-3K/Akt signaling pathways. Accordingly, OSM expression is up-regulated in liver tumours of wt mice and correlates with F4/80 suggesting an interplay between OSM and macrophages recruitment/functions. In particular, wt mice treated with the DEN-CDAA protocol show a stronger correlation between OSM transcript levels and M2 macrophage markers compared with M1 markers. As OSMRβ is fundamental for the activation of the OSM-related STAT3 and PI-3K/Akt signaling pathways, the OSMRβ$^{-/-}$ murine model was employed. In this respect, the data obtained in the OSMRβ$^{-/-}$ murine models shows that the livers of these animals develop smaller tumours. This event is associated to: i) a decreased amount of M2 Tumor Associated Macrophages (TAMs) in the nodules as revealed by the reduction of transcription levels of *CD163, PDL1, CD206* and *CCR2* compared with wt mice; ii) a impairment of the angiogenic process as shown by lower transcript levels of *VE-cadherin, VEGFR2, CD105* and protein level of VEGF, compared with wt mice; iii) a reduction of tumor growth demonstrated by decreased levels of proliferation markers (PCNA, Ki67).

Conclusion: Experimental data highlight a pro-carcinogenic contribution for OSM in NASH, by promoting pro-tumorigenic inflammation, suggesting a possible role for the OSM-OSMRβ axes as therapeutic target for NASH-related HCC.

P10-19
SIRT7 controls the sensitivity of liver cancer to sorafenib through the regulation of ERK Phosphorylation

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Background and Aims:
Sorafenib, a FDA-approved oral multi-kinase inhibitor, improves the prognosis of patients with advanced hepatocellular carcinoma (HCC) in clinical studies, however its resistance restricts the response rate. However, little is known about the underlying molecular process that leads HCC to develop sorafenib resistance. Based on patient-derived data, we recently discovered that SIRT7 could be implicated in this process, and we put forth an effort to clarify how SIRT7 is involved in this occurrence.

Method:
In the Cancer Genome Atlas (TCGA) HCC data, a comparison of the two groups with different prognoses despite Sorafenib treatment, as well as a comparative examination of the transcriptomes of HCC cells presenting Sorafenib resistance, were undertaken to determine the possibility of SIRT7 participation. qRT-PCR and western blotting were used to detect SIRT7 expression in two sorafenib-resistant cell lines, Huh7 sorafenib resistance cells (Huh7^{SR}) and SK-Hep1 sorafenib resistance cells (SK-Hep1^{SR}). The cytotoxic impact of shRNA-mediated SIRT7 silencing and SIRT7 pharmacologic inhibitors whose suppression synergized with sorafenib was tested using cell viability and proliferation assays. In addition, we investigated the function of SIRT7 and the efficiency of SIRT7 pharmacologic inhibitors in a sorafenib-resistant hepatoma cell in vivo xenograft model.

Results:
SIRT7 expression is increased in sorafenib-resistant liver cancer cells, and suppression of SIRT7 results in synthetic lethality when combined with sorafenib therapy. Furthermore, we show that ERK hyperactivation is regulated by SIRT7-mediated deacetylation of DDX3X, and that inhibiting SIRT7 with sorafenib significantly reduced cell survival in vitro and tumor development in vivo. SIRT7 inhibition in combination with sorafenib might successfully restore sorafenib sensitivity. SIRT7 regulates sorafenib resistance via ERK activation and DDX3X deacetylation.

Conclusion:
SIRT7 is responsible for the sorafenib resistance, and its inhibition is most likely to be beneficial together with sorafenib, which can be associated with ERK hyperactivation. The combination therapy identified here may represent a promising strategy for treating advanced HCC in sorafenib-resistant patients.

P11-05-YI
Therapeutic potential of targeting protein hyper-SUMOylation in cholangiocarcinoma

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Background and Aims: cholangiocarcinoma (CCA) comprises a heterogeneous group of malignant tumors with dismal prognosis. Alterations in post-translational modifications (PTMs), including SUMOylation, result in abnormal protein dynamics, cell disturbances and disease. Here, we investigate the role of SUMOylation in CCA development and progression.

Method: levels and function of SUMOylation, together with response to S-adenosylmethionine (SAMe) and ML792 (SUMOylation inhibitors) or CRISPR/Cas9 against *UBE2I* were evaluated *in vitro*, *in vivo* and/or in patients with CCA. The impact of SUMOylation in CCA cells on tumor-stroma crosstalk was assessed performing co-culture experiments with CCA-derived cancer-associated fibroblasts (CAFs), human endothelial cells and monocytes. Proteomic analyses were carried out by mass spectrometry.

Results: the SUMOylation machinery was found overexpressed and overactivated in human CCA cells and tumors, correlating with poor prognosis. Most SUMOylated proteins found upregulated in CCA cells, after SUMO1-immunoprecipitation and further proteomics, participate in cell proliferation, survival or cell homeostasis. Genetic (CRISPR/Cas9-*UBE2I*) and pharmacological (SAMe and ML792) inhibition of SUMOylation reduced CCA cell proliferation and impeded colony formation *in vitro*. Moreover, both SAMe and ML792 induced apoptotic cell death in CCA cells *in vitro*. SUMOylation depletion (SAMe, ML792 or CRISPR/Cas9-*UBE2I*) halted tumorigenesis in subcutaneous models of CCA *in vivo*. Furthermore, SUMOylation deficiency in CCA cells reduced cancer-associated fibroblast and endothelial cell proliferation and impaired macrophage polarization towards an anti-inflammatory M2-like phenotype.

Conclusion: aberrant protein SUMOylation contributes to cholangiocarcinogenesis by promoting cell survival and proliferation. Moreover, SUMOylation impacts the CCA-stroma crosstalk. Impaired SUMOylation halts CCA growth and, thus, may represent a potential new therapeutic strategy for patients with CCA.

P11-06-YI
Cyclin M1, a novel therapeutic target for epithelial-mesenchymal transition in Hepatocellular Carcinoma

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Background and Aims: Hepatocellular carcinoma (HCC) is the sixth most commonly occurring cancer and the second cause of cancer mortality (Katherine A. McGlynn, 2015). In USA, the prognosis has an overall 5-years survival of approximately 14% (Katherine A. McGlynn, 2015). A decrease of magnesium (Mg^{2+}) levels is associated with the progression and metastasis of HCC, however the mechanisms for Mg^{2+} dysregulation remain poorly understood (Liu M, 2019). Considering the alteration in Mg^{2+} levels in HCC and the role of Mg^{2+} transporters to allow its flux across cell membranes. Herein, we have studied the role of the Mg^{2+} transporter Cyclin M1 (CNNM1) in the epithelial-mesenchymal transition (EMT) in HCC.

Method: CNNM1 mRNA expression levels were measured in a cohort of patients with HCC. We performed an in silico analysis of CNNM1 and mesenchymal markers expression levels by cbioportal website in 450 patients. In the preclinical models, we measured CNNM1 levels in mice treated with diethylnitrosamine (DENA)- induced HCC for 5, 8 and 12 months. In the in vitro studies, we measured CNNM1 levels and cytosolic Mg^{2+} levels in human epithelial and mesenchymal cells. We also studied CNNM1 expression levels in hep3b cell line treated with the mesenchymal modulator Tgfβ.

Results: A cohort of patients with HCC presented an upregulation of CNNM1 mRNA expression levels. Moreover, an in silico analysis by cbioportal revealed an increase of CNNM1 levels in patients with tumors in T1 stage, whereas its expression is reduced in patients with tumors in advanced stage. In the preclinical models, Cnnm1 mRNA levels significantly increased in mice treated with diethylnitrosamine (DENA)-induced HCC for 5, 8 and 12 months. This outcome was consistent with the observations in the cohort of HCC patients.

In the in vitro studies, we noticed a discrepancy between CNNM1 protein and mRNA expression levels in human epithelial and mesenchymal cells. Indeed, the same controversy was found in Hep3b cell line treated with Tgfβ. These results suggest a regulation of CNNM1 levels in the EMT. As CNNM1 is a Mg^{2+} transporter, we measured cytosolic Mg^{2+} levels in human EMT cell lines. The mesenchymal cell lines, which possess high expression of CNNM1 at protein levels, present lower levels of Mg^{2+} than the epithelial cell lines, suggesting that CNNM1 acts as a Mg^{2+} extruder.

Conclusion: CNNM1 may be a crucial factor in the EMT in HCC through modulating Mg^{2+} metabolism.

P11-10
Identifying and analyzing metabolic reprogramming in HCC

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Background and Aims: Metabolic reprogramming is a key hallmark of cancers, including hepatocellular carcinoma (HCC). Understanding how metabolism changes as tumors develop is a crucial step towards identifying novel and more effective therapeutic targets against HCC. **Aims:** (1). Analyze the dynamics of metabolic reprogramming in HCC at different stages of tumor development, regression and recurrence. (2). Determine the effects of therapeutic modulation of metabolism-associated non-coding microRNAs on HCC development. We have analyzed the underlying mechanisms and functional relevance of cancer-associated microRNAs in metabolic reprogramming, during multiple stages of liver tumor progression and regression. We have determined the effects of their modulation on HCC development.

Method: Global metabolomics analyses on liver tissues at different stages of tumor development, regression and recurrence, in an oncogene-driven conditional mouse model of HCC was done. This was followed by in-depth *in silico* analyses. Metabolic flux was studied for metabolic characterization of HCC, using serial multi-tracer PET/CT on two HCC mouse models. 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 identified distinct metabolic profiles during the different stages of HCC development, regression and recurrence. Further, our serial PET/CT analyses showed that metabolic changes were distinct not only between the different stages of HCC, but also between the different oncogene-dependent mouse models. Therapeutic targeting of specific miRNAs associated with metabolism, led to significant attenuation of HCC development in these models.

Conclusion: Metabolic reprogramming starts very early in HCC development. It is a very dynamic process, significantly and distinctly changing as tumors develop, regress and even recur. Elucidating these changes will help identify specific switches and targets for therapeutic intervention in HCC. Non-coding RNAs that regulate specific targets in metabolic pathways, may be therapeutically modulated to affect tumor development.

P11-12
Reduced expression of YAP is associated with transition of pre-invasive to invasive state in an organoid-derived model of cholangiocarcinoma

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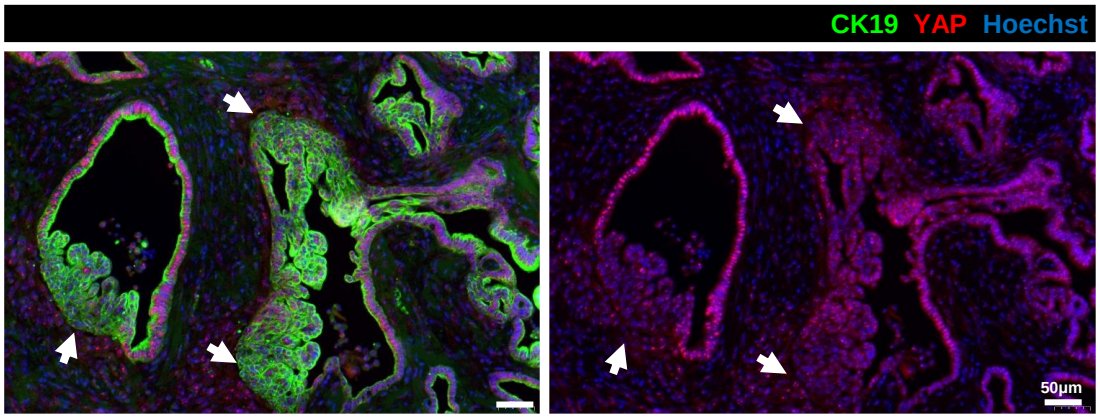
Background and Aims: Cholangiocarcinoma remains associated with poor prognosis, in part because of late diagnosis resulting from limited symptoms and from insufficient knowledge on the early stages of the disease. Therefore, our aim is to uncover how cholangiocytes develop into pre-cancerous lesions and subsequently into malignant invasive tumours.

Method: We generated organoids from mouse cholangiocytes that carry the *Kras*^{G12D} mutation. The organoids were studied *in vitro* and *in vivo* after injection in immune-depressed mice. Tumour formation and progression were investigated by characterising the transcriptome and the histo-morphological features of the epithelial cells and surrounding stroma.

Results: Expression of *Kras*^{G12D} in organoids had no morphological impact *in vitro* as compared to wild-type organoids. However, *Kras*^{G12D} organoids, but not wild-type organoids, induced tumours when injected subcutaneously in mice. Like in cholangiocarcinoma, the tumours were desmoplastic and the epithelia delineated cystic structures which were predominantly lined by a monolayered cholangiocyte epithelium. The epithelium also displayed transitional areas with loss of polarity, multilayer formation, loss of epithelialisation, and invasion through the basal lamina. These transitional areas showed reduced expression of YAP, an effector of Hippo signalling. Furthermore, *Kras*^{G12D}-organoids treated *in vitro* with a YAP inhibitor developed features of epithelial-mesencymal transition, suggesting that YAP inhibition contributes to tumour progression by modulating the epithelial character of the cells. Finally, RNA-Seq of wild-type and *Kras*^{G12D} organoids grown *in vitro* revealed gene candidates that trigger tumorigenesis.

Conclusion: We have developed an original and versatile model to study the transition of precancerous cells towards an invasive cholangiocarcinoma phenotype, and provide evidence that Hippo signalling plays a role in this transition. Future work will focus on the role of YAP and of genes that are differentially expressed in tumorigenic *Kras*^{G12D}-organoids, and on the role of the extracellular matrix in the areas where cells transit to an invasive state.

Figure: Immunofluorescence image of a *Kras*^{G12D} organoid-derived tumor displaying transitional monolayer/multilayered epithelial (CK19^{+}) structures where YAP expression is reduced (arrows).



P11-13
Neutrophil degranulation and ageing as potential therapeutic targets in hepatocellular carcinoma?

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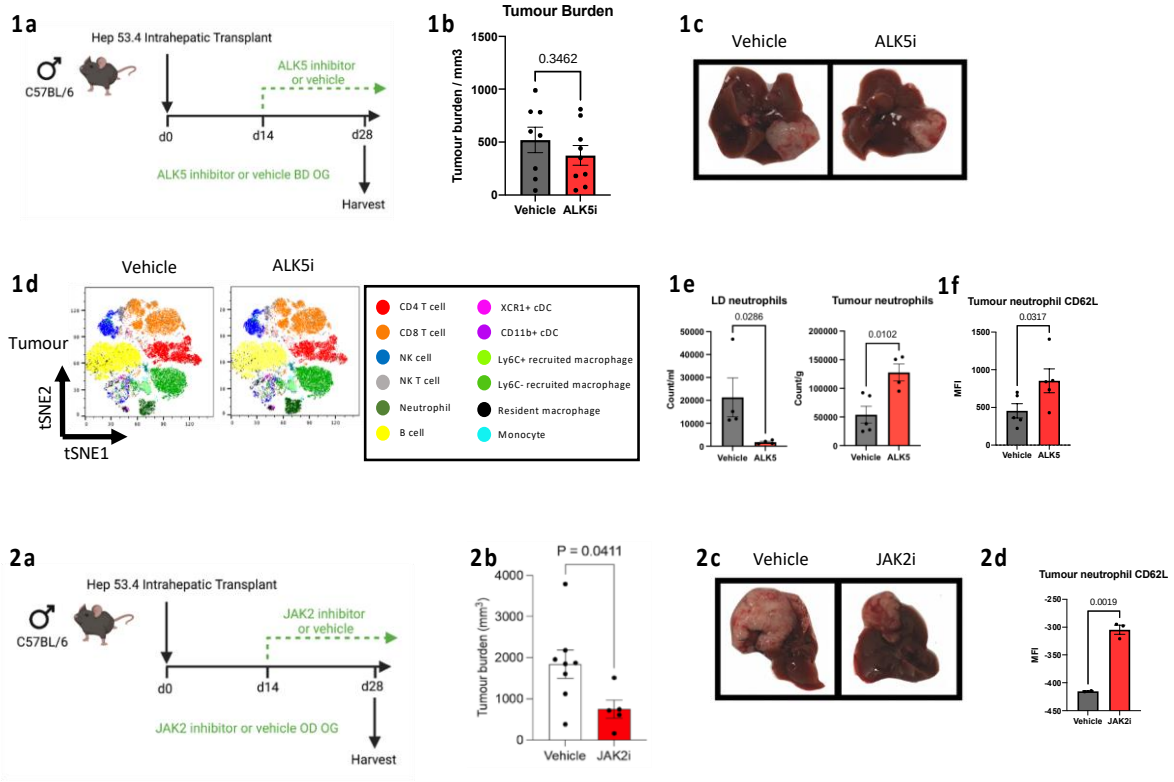
Background and Aims: Neutrophils are recognised to play a vital role in hepatocellular carcinoma (HCC) progression through pro-tumour functions such as creating an immunosuppressive tumour microenvironment. This makes them potential therapeutic targets. Aims; 1. To characterise neutrophil phenotype and heterogenicity in HCC using patient samples and an *in vivo* model. 2. Manipulate HCC specific neutrophil changes in our *in vivo* model in order to develop novel neutrophil directed therapies for HCC.

Method: *Patient study.* Circulating neutrophils were isolated from HCC and chronic liver disease patients using density centrifugation. *In vivo model.* C57BL/6J mice underwent orthotopic tumour implantation. Both patient and mouse neutrophils were characterised using flow cytometry and functional assays.

Results: HCC patient blood samples were identified as having an increase in low density (LD) CD16^{high} neutrophils compared to healthy controls. Flow cytometry analysis revealed LD CD16^{hi} neutrophils as being more activated, degranulated and aged compared to normal density (ND) neutrophils as demonstrated by differing expression of CXCR2, CXCR4, CD11b, CD62L, CD10 and CD66b. In addition, LD CD16^{hi} neutrophils expressed markers associated with pro-tumour neutrophils such as CD36 and LOX-1. Tumour bearing mice also developed an increase in LD neutrophil frequency compared to controls with their phenotypic features being highly conserved compared to human. Functionally LD neutrophils were less phagocytic, had reduced migration to CXCL2 and had increased basal reactive oxygen species (ROS) production but a blunted stimulated ROS response further supporting them as being aged and degranulated neutrophils. This highlights circulating LD neutrophils and neutrophil ageing and degranulation as potential therapeutic targets. We used TGF-β and JAK2/STAT3 inhibition in our in vivo model in order to test this. ALK5 (TGF-β receptor 1) inhibition failed to significantly impact tumour burden however did significantly reduce the frequency of circulating LD neutrophils, increase the frequency of tumour associated neutrophils and increase CD62L expression indicating reduced ageing/degranulation (figure 1a-f). JAK2 inhibition significantly reduced tumour burden and altered neutrophil phenotype with an increase in neutrophil CD62L expression (figure 2a-d).

Conclusion: We have identified an increase in frequency of circulating LD neutrophils with a degranulated, aged and pro-tumour phenotype in HCC that are highly conserved between HCC patients and tumour bearing mice. Inhibition of the TGF-β and JAK2/STAT3 pathway using ALK5 and JAK2 inhibitors resulted in alterations of neutrophil phenotype suggesting reduced degranulation and ageing. Further studies investigating these therapeutic agents in combination with immune checkpoint inhibitors is warranted.

Figure:


1a
Hep 53.4 Intrahepatic Transplant
C57BL/6
ALK5 inhibitor or vehicle
d0
d14
d28
ALK5 inhibitor or vehicle BD OG
Harvest
1b
Tumour Burden
Tumour burden / mm3
0.3462
Vehicle
ALK5i
1c
Vehicle
ALK5i
1d
Vehicle
ALK5i
Tumour
tSNE2
tSNE1
CD4 T cell
CD8 T cell
NK cell
NK T cell
Neutrophil
B cell
XCR1+ cDC
CD11b+ cDC
Ly6C+ recruited macrophage
Ly6C- recruited macrophage
Resident macrophage
Monocyte
1e
LD neutrophils
0.0286
Tumour neutrophils
0.0102
Vehicle
ALK5
1f
Tumour neutrophil CD62L
0.0317
MFI
2a
Hep 53.4 Intrahepatic Transplant
C57BL/6
JAK2 inhibitor or vehicle
d0
d14
d28
JAK2 inhibitor or vehicle OD OG
Harvest
2b
P = 0.0411
Tumour burden (mm3)
Vehicle
JAK2i
2c
Vehicle
JAK2i
2d
Tumour neutrophil CD62L
0.0019
MFI
Vehicle
JAK2i

POSTER ABSTRACT PRESENTATIONS

Clinical Science

P01-01
Atezolizumab in combination with bevacizumab in patients with unresectable hepatocellular carcinoma not previously treated with systemic therapy: safety results from the interim analysis of the phase IIIb Italian AMETHISTA trial

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Background and Aims: AMETHISTA is a prospective, open-label, single-arm, phase IIIb trial conducted in 21 Italian centres, which evaluates the safety of atezolizumab (atezo) plus bevacizumab (bev) in patients (pts) with unresectable HCC. An interim analysis has been conducted approximately at one year from the end of enrolment.

Method: The trial included pts with histologically confirmed HCC not amenable to surgery and/or locoregional therapies, no previous systemic therapy for HCC, ECOG PS 0-1, Child Pugh class A. If present, varices were to be treated per local standard of care prior to enrolment. Atezo 1200 mg/bev 15 mg/kg were given IV Q3W until unacceptable toxicity or loss of clinical benefit. The primary safety endpoint was the incidence of grade 3-5 NCI CTCAE v.5 bleedings/haemorrhages.

Results: 152 pts were enrolled and 149 (118 males, median age 69 years) were treated. At the cut-off date, the median follow-up was 13.4 months, 50 patients (32.9% of enrolled) were still on treatment and 99 (65.1%) permanently discontinued treatment. 63 patients (41.5%) discontinued the study and 36

(23.7%) were in follow-up. The median exposures to atezo and bev were 8.3 and 7.3 months (12.0 and 11.0 cycles), respectively. Overall, 44 pts (29.5%) permanently discontinued treatment due to AEs (discontinuation of atezo in 32 patients, 21.5%, and of bev in 42, 28.2%). 21 Grade 3-5 bleeding/haemorrhage events occurred in 17 pts (11.4%). The table shows data of grade 3-5 bleedings/haemorrhages, treatment-emergent adverse events (TEAE), serious TEAE, fatal TEAE and adverse events of special interest (AESI) overall and by relationship to treatment. Grade 3-4 TEAE were reported in 90 (60.4%) pts and were treatment-related in 60 (40.3%). Overall, 106 pts (71.1%) had treatment-related TEAEs: the most common were hypertension (39 patients, 26.2%), pruritus (17 patients, 11.4%) and asthenia (16 patients, 10.7%). The most common serious TEAEs were pulmonary embolism (bev-related in 4 patients) and sepsis (not related to any drug), both in 5 patients, 3.4%.

Conclusion: This interim analysis of safety data from the AMETHISTA trial at a median follow up of 13.4 months showed that the incidence of grade 3-5 bleedings/haemorrhages was in line with that previously reported in the IMbrave150 trial. The safety findings in a population of unresectable HCC pts similar to that usually found in clinical practice were consistent with the known safety profile of atezo and bev.

Figure:

	Overall	Related to atezolizumab	Related to bevacizumab	Related to both
Grade 3-4 bleeding/haemorrhage	14(9.4%)	1 (0.7%)	9(6.0%)	0 (0.0%)
Grade 5 bleeding/haemorrhage	3 (2.0%)	0 (0.0%)	1 (0.7%)	0 (0.0%)
TEAE	143 (96.0%)	86 (57.7%)	91 (61.1%)	51 (34.2%)
Serious TEAE	53 (35.6%)	8 (5.4%)	24 (16.1%)	5 (3.4%)
Fatal TEAE	14 (9.4%)	0 (0.0%)	3 (2.0%)	0 (0.0%)
AESI	57 (38.3%)	15 (10.1%)	35 (23.5%)	4 (2.7%)

Data are n (%) of patients in the safety population (149 pts)

P01-02
Regorafenib in patients with unresectable hepatocellular carcinoma in real-world practice: Final analysis of the prospective, observational REFINE study in the sorafenib-intolerant patient subgroup

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Background and Aims: In the phase 3 RESORCE study, regorafenib significantly improved overall survival in patients (pts) with unresectable hepatocellular carcinoma (uHCC) who tolerated (≥ 400 mg/day for ≥ 20 of the last 28 days of treatment) and progressed on sorafenib (SOR) and had Child–Pugh A liver status. The observational REFINE study evaluated a broader population than RESORCE, including pts with Eastern Cooperative Oncology Group (ECOG) performance status ≥ 2, Child–Pugh B status, and SOR intolerance. Here, we present the final analysis of SOR-intolerant pts vs the overall cohort in REFINE.

Method: REFINE (NCT03289273) was an international, prospective, multicenter study that enrolled pts with uHCC for whom the decision to treat with regorafenib was made by their physician before enrollment, according to the local health authority approved label. The primary aim was safety, including incidence of treatment-emergent adverse events (TEAEs) (MedDRA v25) and dose modifications due to TEAEs. Pts were considered SOR intolerant if they experienced TEAEs leading to SOR discontinuation but still went on to receive regorafenib treatment.

Results: Overall, 1005 pts initiated regorafenib and were eligible for analysis. Of the 965 pts who had received prior SOR, 91 (9%) were SOR intolerant. At baseline, median age of SOR-intolerant pts was 65 years (range 34–89), and 77% were male. A higher proportion of SOR-intolerant pts were Child–Pugh B at study entry vs the overall cohort (19% vs 12%; **Table**). Nearly half (49%) of SOR-intolerant pts initiated regorafenib at 80 mg/day. Overall, 93% of SOR-intolerant pts experienced a TEAE of any grade. A similar rate of drug-related TEAEs was reported in SOR-intolerant pts vs the overall cohort (74%, both). The most common drug-related TEAEs (any grade, >10%) in SOR-intolerant pts were diarrhea (25%), hand–foot skin reaction (HFSR; 25%), asthenia (16%), decreased appetite (14%), and hypertension (11%). In SOR-intolerant pts the incidence and severity of drug-related TEAEs, including diarrhea and HFSR, were similar to the overall cohort, and none were grade 4 or 5.

Conclusion: In REFINE, despite the evaluation of a broad population of real-world pts with uHCC, there were no new safety signals associated with regorafenib. The safety profile of regorafenib in SOR-intolerant pts showed no difference to that in the overall cohort.

Figure:

n (%)	SOR intolerant subgroup (n = 91)	All pts (N = 1005)
ECOG performance status[a]		
0/1	76 (84)	829 (82)
≥2	5 (5)	60 (6)
Child–Pugh class[a]		
A	48 (53)	618 (61)
B	17 (19)	123 (12)
C	0	5 (<1)
Barcelona Clinic Liver Cancer stage[a]		
B	15 (16)	133 (13)
C	53 (58)	625 (62)
Other	3 (3)	34 (3)
HCC etiology (multiple responses)[a,b]		
Alcohol use	27 (30)	250 (25)
Hepatitis C virus	24 (26)	242 (24)
Hepatitis B virus	20 (22)	382 (38)
Nonalcoholic steatohepatitis	7 (8)	66 (7)
[a]Missing/unknown or non-evaluable data not shown. [b]Excluding genetic/metabolic and other.		

P01-03
Glypican-3 targeted radiopharmaceutical therapy for hepatocellular carcinoma: Preclinical characterization of a novel peptide binder

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Background and Aims: Glypican-3 (GPC3) is a membrane-associated heparan sulfate proteoglycan primarily involved in embryonic development, and is barely detectable in normal adult tissues. Significant upregulation of GPC3 protein in hepatocellular carcinomas (HCC) has been observed in multiple immunohistochemistry (IHC) studies with up to 75% positivity rate, and is associated with poor prognosis. GPC3 is not expressed in healthy or non-malignant liver tissue. Targeting GPC3 could fulfill an unmet medical need for HCC, a leading cause of cancer-related deaths worldwide for which efficacious therapies are lacking. The differential expression of GPC3 between tumor and normal tissues provides an opportunity for GPC3-targeted radiopharmaceutical therapy (RPT) to treat HCC.

Method: RAYZ-8009 is comprised of a novel macrocyclic peptide binder to GPC3, a linker, and chelator DOTA that can be complexed with different radioisotopes. The affinity of peptide binders to GPC3 was determined by surface plasma resonance (SPR), as well as a radioligand binding assay in human HCC cell line HepG2. The cross-species binding was assessed by radioligand binding using recombinant mouse, cynomolgus monkey, and human GPC3 proteins. Target-mediated internalization in HepG2 cells was measured using Microbeta at various time points. In vivo biodistribution with ^{177}Lu, and anti-tumor efficacy studies using ^{225}Ac were performed in HepG2 tumor-bearing athymic nude mice.

Results: RAYZ-8009 showed high binding affinity to human GPC3 with a K_D of 0.7 nM as determined by SPR. Binding affinity was maintained across mouse, cynomolgus monkey and human GPC3. Potent cellular binding was confirmed in GPC3+ HepG2 cells, and was independent of choice of isotope. ^{177}Lu-RAYZ-8009 showed fast and efficient internalization with 42% internalized by 20 minutes in HepG2 cells. In vivo biodistribution of ^{177}Lu-RAYZ-8009 showed tumor uptake of 19.8, 16.6, 16.4, and 8.8 %ID/g at 2, 24, 48, and 96 hours, respectively. Renal uptake was 16.1, 4.7, 1.6, and 0.7 %ID/g at the same timepoints, with tumor/kidney ratios of 1.3, 3.7, 11.3, and 15.0, respectively. Minimal uptake was observed in other normal tissues. Tumor-specific uptake and retention were also observed when tumors were implanted orthotopically with no uptake in non-malignant liver tissue. Furthermore, significant tumor growth inhibition and survival benefit were achieved with ^{225}Ac-RAYZ-8009 in GPC3+ HCC xenografts.

Conclusion: Preclinical pharmacodynamic, pharmacokinetic, biodistribution and efficacy data demonstrate the potential of RAYZ-8009 as a RPT agent for the treatment of patients with GPC3-positive HCC.

P01-04-YI
Barriers to hepatocellular carcinoma screening at a single Veterans Affairs center: a quality improvement initiative

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Background and Aims: Hepatocellular carcinoma (HCC) is the most common cause of morbidity and mortality in patients with cirrhosis. Guidelines from prominent liver societies worldwide recommend HCC screening with abdominal ultrasound ± serum alpha-fetoprotein every 6 months in all patients with cirrhosis and certain ones with chronic hepatitis B. In 1385 eligible Veterans this past year, HCC screening remains suboptimal at 38% at our center. The goal of this project was to identify barriers to HCC screening at our Veterans Health Administration center.

Method: Direct observations, process mapping, and semi-structured interviews were used for process analysis. The investigator observed gastroenterology (GI) clinicians and sonographers and interviewed Veterans, GI/ primary care providers (PCPs), scheduling assistants, a radiologist, and sonographers. Fishbone and process maps were refined from these observations and interviews. Problem analysis was conducted through an advanced liver disease (ALD) dashboard that tracks cirrhotic patients overdue for HCC screening. A pareto chart was created from chart review of 30 veterans who were overdue for HCC screening at the 5 lowest performing sites.

Results: The process map showed the complexity of the screening process across different departments involved. Patient-level barriers included distance to the center, transportation, knowledge about risk factors and benefits of screening, and difficulty in scheduling imaging. Provider-level barriers included lack of knowledge about risk factors and benefits of screening, omission of cirrhosis diagnosis in active problems, and lack of time by PCP to address cirrhosis. System-level barriers included prolonged radiology wait times, no identified owner (PCP vs. GI) of the screening process, difficulty scheduling ultrasounds due to phone tags and wrong contact information, lack of trained staff to perform imaging, and no standardized way to track screening done outside of the medical center. Analysis of the ALD dashboard showed that 78% (n = 385) of veterans seen at GI/ hepatology clinics were up to date with HCC screening, while only 23% (n = 990) were if they were not seen at the GI/hepatology clinics. A pareto chart demonstrated that the most common cause of overdue HCC screening was that screening was not ordered. Follow-up interviews revealed that most PCPs believe that HCC screening should be owned by the GI/ hepatology clinic due to competing priorities.

Conclusion: Barriers to HCC screening were identified using quality improvement (QI) tools like fishbone diagrams, process maps and pareto charts. The findings led to the creation of an outreach program with patient navigators contacting veterans via phone and mail and ordering ultrasounds. A QI team was formed with Plan-Do-Study-Act cycles planned to improve overall HCC screening rates from 38% to 48% within a 3-month span.

P01-05-YI
Predicting cardiovascular risk in patients with hepatocellular carcinoma receiving anti-angiogenic drugs: lessons from sorafenib

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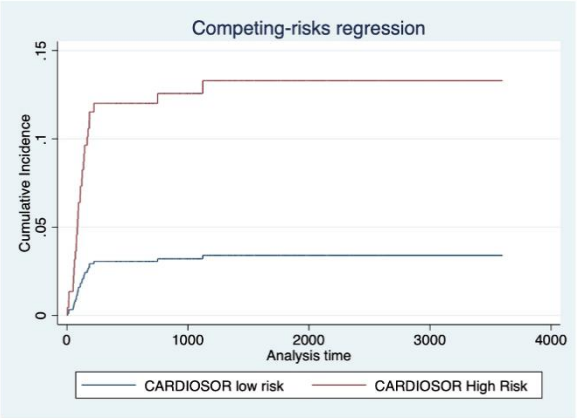
Background and Aims: Antineoplastic agents targeting the VEGF-VEGFR pathway increase the risk of major adverse cardiac and cerebrovascular events (MACE). In the HCC setting these agents include bevacizumab (as part of the first-line combination treatment) and TKIs. The European Society of Cardiology proposed a risk stratification algorithm for cardio-oncology (ESC-2022), never been tested in HCC. The CARDIOSOR score has been also proposed (Carballo-Folgoso, 2021) but lacks external validation in predicting MACE in sorafenib-treated patients.

Method: Retrospective analysis of the ARPES and ITA.LI.CA databases to test the ESC-2022 and CARDIOSOR abilities in predicting MACE in sorafenib-treated HCC patients (2010-2018 timeframe). Evolutive events after sorafenib start (including the occurrence of MACE) were available for all patients. Competing-risk regressions for each score were performed to address the study aim.

Results: This study included 815 patients, 28 suffered at least one MACE (3.4%). The four-tier ESC classification showed an sHR 1.42, (p = 0.015) per every risk-class (1-year risk 1.7%, 2.1%, 4.3%, and 8.0% in the low, medium, high, and very-high-risk tiers, respectively). The dichotomous CARDIOSOR scale identified a high-risk group with a 4-fold increased risk of MACE (sHR 4.12, p= 0.007; 1-year risk 3.2% and 13.1%). Both score had similar predictive ability Akaike information criterion 360 and 366, respectively.

Conclusion: The risk of MACE in patients receiving TKI for HCC was non-negligible. Both scores discriminated this probability accurately, with different perks and pitfalls. These tools will be useful in the near-future to identify high-risk patients, candidate for anti-VEGF-free regimens (i.e. tremelimumab-durvalumab).

Figure:


Competing-risks regression
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.15
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4000
Analysis time
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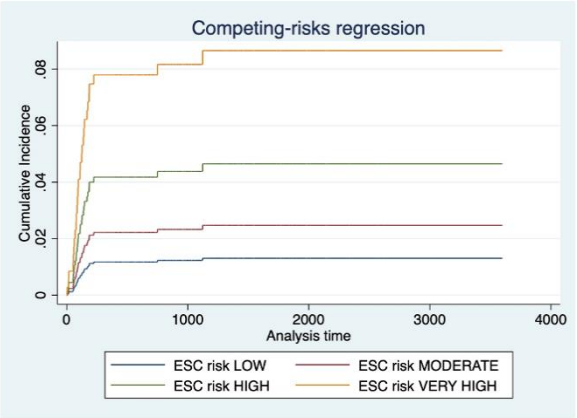
CARDIOSOR High Risk


Competing-risks regression
Cumulative Incidence
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.04
.06
.08
0
1000
2000
3000
4000
Analysis time
ESC risk LOW
ESC risk MODERATE
ESC risk HIGH
ESC risk VERY HIGH

P01-06-YI
Survival outcomes from Atezolizumab plus Bevacizumab versus Lenvatinib versus Sorafenib in Child Pugh B unresectable hepatocellular carcinoma patients

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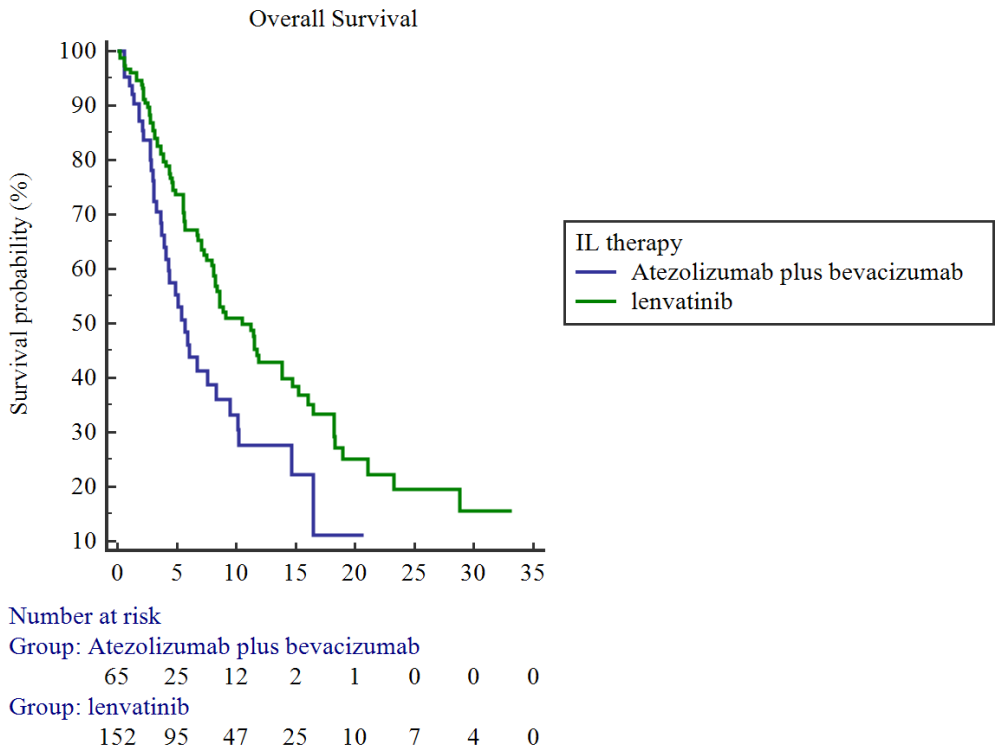
Background and Aims: The best first line treatment for patients with advanced hepatocellular carcinoma (HCC) and Child-Pugh (CP) class B remains unknown. The aim of the present study was to perform a real-world analysis on a large sample of patients with unresectable HCC with CP B treated with atezolizumab plus bevacizumab Vs Lenvatinib.

Method: The study population included patients affected by advanced (BCLC-C) or intermediate (BCLC-B) HCC patients not suitable for locoregional therapies from both Western and Eastern world (Italy, Germany, Republic of Korea and Japan), who received atezolizumab plus bevacizumab or Lenvatinib as first line treatment. All the study population presented a CP class of B.
The primary endpoint of the study was overall survival (OS) of CP B patients treated with Lenvatinib compared to atezolizumab plus bevacizumab. Survival curves were estimated using the product-limit method of Kaplan-Meier. The role of stratification factors was analyzed with log-rank tests.
Finally, an interaction test was performed for the main baseline clinical characteristics.

Results: 217 CP B HCC patients were enrolled in the study: 65 (30%) received atezolizumab plus bevacizumab, and 152 (70%) received lenvatinib.
The mOS for patients receiving Lenvatinib was 13.8 months (95% CI: 11.6-16.0), compared to 8.2 months (95% CI 6.3-10.2) for patients receiving atezolizumab plus bevacizumab as first line treatment (atezolizumab plus bevacizumab Vs Lenvatinib: HR 1.9, 95% CI 1.2-3.0, p=0.0050). No statistically significant differences were highlighted in terms of mPFS. The multivariate analysis confirmed that patients receiving Lenvatinib as first line treatment has a significantly longer OS compared to patients receiving atezolizumab plus bevacizumab (HR 2.01; 95% CI 1.29-3.25, p=0.0023). By evaluating the cohort of patients who received atezolizumab plus bevacizumab, we found that Child B patients with ECOG PS 0, or BCLC B stage or ALBI grade 1 were those who had benefit from the treatment thus showing survival outcomes no significantly different compared to those receiving Lenvatinib.

Conclusion: The present study suggests for the first time a major benefit from Lenvatinib compared to atezolizumab plus bevacizumab in a large cohort of patients with CP B class HCC.

Figure:


Overall Survival
Survival probability (%)
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
35
IL therapy
Atezolizumab plus bevacizumab
lenvatinib
Number at risk
Group: Atezolizumab plus bevacizumab
65 25 12 2 1 0 0 0
Group: lenvatinib
152 95 47 25 10 7 4 0

P01-11
Tolerability of first line systemic therapy in elderly patients with advanced hepatocellular carcinoma

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Background and Aims: Hepatocellular carcinoma (HCC) accounts for 90% of primary liver cancer. The average age of HCC development is 70, with aging being a known risk factor. Elderly patients should not be excluded from systemic therapy based upon age alone and all available treatments can be recommended for them.

Method: We considered 126 patients affected by advanced HCC, treated with first line systemic therapy. Four patients were excluded from the final analysis due to lack of follow-up (FU) information, 6 patients on atezolizumab/bevacizumab therapy were excluded for less than 3 months of FU at the time of data collection. We studied patients' overall survival (OS), time to progression (TTP) represented as therapy duration and adverse events (AE) secondary to two systemic therapies, namely sorafenib (SB) and lenvatinib (LB).

Results: Patients were predominantly men (80.3%); 84.6% of them suffered from cirrhosis, which the most frequent etiology was hepatitis C (44.4%). Thirty-nine percent of patients carried steatosis and metabolic syndrome. Median age at diagnosis of HCC was 72 [27-88], median age at systemic therapy start was 73 [28-88]. Patients older than 65 years represented the 80.2% of our cohort, over 70 were the 42.2%, while over 80 were 19.8% of the total. The median alpha-fetoprotein value before initiation of therapy was 45 [1.2-83000]. Twenty-six patients were treated with LB and median age at therapy start was 76, 90 patients were treated with SB, median age at therapy start was 72.5 ($p = 0.04$). Median systemic therapy duration was 11 months in patients treated with LB, 4 months in patients treated with SB ($p < 0.05$). Median OS was 18.8 months in SB group and 52.7 months in LB group ($p < 0.05$). No difference was observed in therapy duration considering age > 80 years ($p = 0.97$). No difference was observed in term of OS considering patients younger or older than 80 years ($p = 0.72$). Most reported AE were fatigue, anorexia and diarrhea, with the latter more common in patients younger than 80 years ($p < 0.05$). Regarding diarrhea, only 4 patients over 80 (17.4%) had a Common Terminology Criteria for Adverse Events grade > 1. Patients over 80 years did not require a dose reduction more than younger ones ($p = 0.97$). Considering the reason for discontinuing therapy, no difference was observed between patients older than or younger than 80 years ($p = 0.70$).

Conclusion: Our study demonstrates how elderly patients could be treated safely with the same intensity as younger ones. AE didn't represent a crucial factor for discontinuing therapy in elderly. It is essential to know how to manage AE in a timely way, educating the patient to recognize them. Knowing that the epidemiology of HCC will increasingly affect elderly patients, the choice of treatment based on the comorbidity and characteristics of the subject will be decisive, but age alone should not represent a limitation at the beginning of systemic therapy.

P01-12
Evaluation of hepatocellular carcinoma and mortality in Budd-Chiari syndrome

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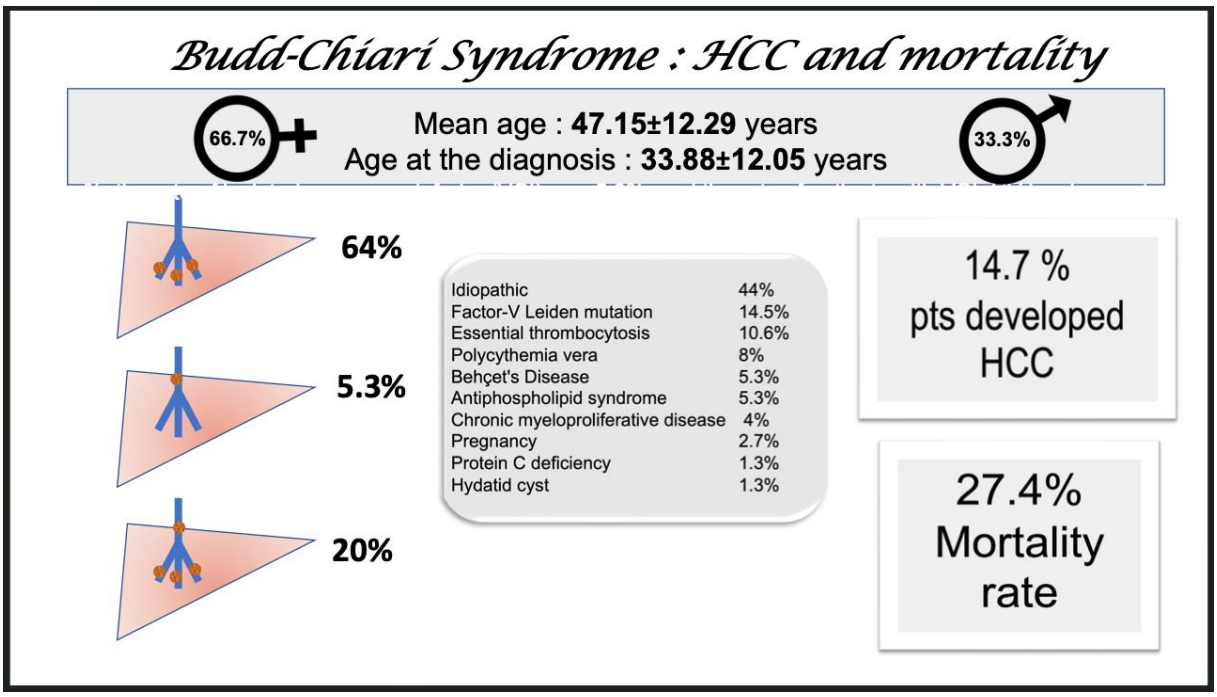
Background and Aims: Different clinical situations may occur, which can range from cirrhosis to hepatocellular carcinoma (HCC), in Budd-Chiari syndrome (BCS). HCC rarely develops in BCS and its pathogenetic mechanisms are not clearly understood yet. In this study, we aimed to evaluate the frequency of HCC in BCS and the mortality rate together with the factors that may be associated with it.

Method: This is a single-center and retrospective study. There were 75 patients who were diagnosed with BCS and followed up outpatient clinic in a tertiary center. Data of the patients were recorded from the patient files. Comparison of measurements according to categorical groups was made with independent t-test. The chi-square test was used to determine the relationships between categorical variables.

Results: The mean age of the patients was 47.15 ± 12.29 years. The age of the patients at the time of diagnosis was 33.88 ± 12.05 years. Fifty (66.7%) of the patients were women. The causes of BCS were idiopathic in 44%, Factor-V Leiden mutation in 14.5%, essential thrombocytosis in 10.6%, polycythemia vera in 8%, Behçet's Disease in 5.3%, antiphospholipid syndrome in 5.3%, chronic myeloproliferative disease in 4%, pregnancy in 2.7%, Protein C deficiency in 1.3%, and hydatid cyst in 1.3%. The rate of patients with only hepatic vein (HV) involvement at the time of diagnosis was 64%, the rate of isolated vena cava inferior (VCI) was 5.3%, and the rate of patients with VCI and HV co-involvement was 20%. The number of patients who developed HCC was 11 (14.7%). Factors likely to contribute to the development of HCC were also evaluated. Patient age (p = 0.784), gender (p = 0.336), and etiology (p = 0.567) were not statistically correlated with the risk of developing HCC, at diagnosis. Patients with and without HCC were similar in terms of demographic characteristics, age, gender, disease duration, patient age, age at diagnosis, and anticoagulant use. The diagnosis of HCC was made by radiological evaluation in 10 patients and with biopsy in one patient. AFP levels of patients with HCC were found to be 400 ng/mL in one patient and within normal limits in the others. The mortality rate in patients who developed HCC was 36%. 4 patients who developed HCC underwent liver transplantation, 3 patients received radiological treatment and one patient received oncological treatment. The overall mortality rate of the patients was 27.4% (n = 17). When factors related to mortality were evaluated, the mortality rate was found to be higher in patients with the coexistence of VCI and HV thrombosis (p = 0.049).

Conclusion: The mortality rate was found to be high in patients with BCS. It has been observed that the co-involvement of VCI and HV and the development of HCC are associated with increased mortality.

Figure:


Budd-Chiari Syndrome : HCC and mortality
66.7%
Mean age : 47.15±12.29 years
Age at the diagnosis : 33.88±12.05 years
33.3%
64%
5.3%
20%
Idiopathic 44%
Factor-V Leiden mutation 14.5%
Essential thrombocytosis 10.6%
Polycythemia vera 8%
Behçet's Disease 5.3%
Antiphospholipid syndrome 5.3%
Chronic myeloproliferative disease 4%
Pregnancy 2.7%
Protein C deficiency 1.3%
Hydatid cyst 1.3%
14.7 % pts developed HCC
27.4% Mortality rate

P01-14
Validation of the CRAFITY score in patients with hepatocellular carcinoma treated with atezolizumab and bevacizumab

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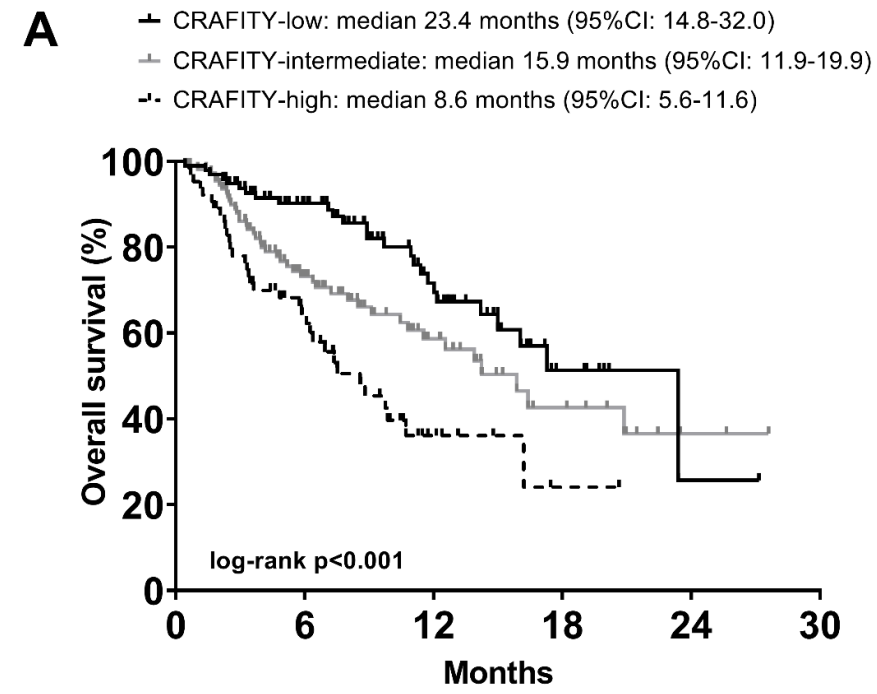
Background and Aims: We recently developed the C-reactive protein (CRP) and alpha-fetoprotein (AFP) in Immunotherapy (CRAFITY) score in patients with hepatocellular carcinoma (HCC) undergoing immune checkpoint inhibitor (ICI) therapy. As CRAFITY was developed in patients undergoing different ICI-based regimens, the score requires validation in patients treated with atezolizumab and bevacizumab (AB), the current standard of care in systemic first-line treatment for advanced HCC.

Method: AB-treated patients with HCC at 15 centers in Europe and Asia between 12/2018 and 01/2023 were included. CRAFITY was derived from serum CRP and AFP values prior to AB initiation by adding one point each for CRP ≥1mg/dL and AFP ≥100ng/mL resulting in the following categories: 0 points = CRAFITY-low, 1 point = CRAFITY-intermediate, 2 points = CRAFITY-high. The prognostic (overall survival (OS) and progression-free survival (PFS)) and predictive ability (best radiological response) of the CRAFITY score were assessed using uni- and multivariable analyses.

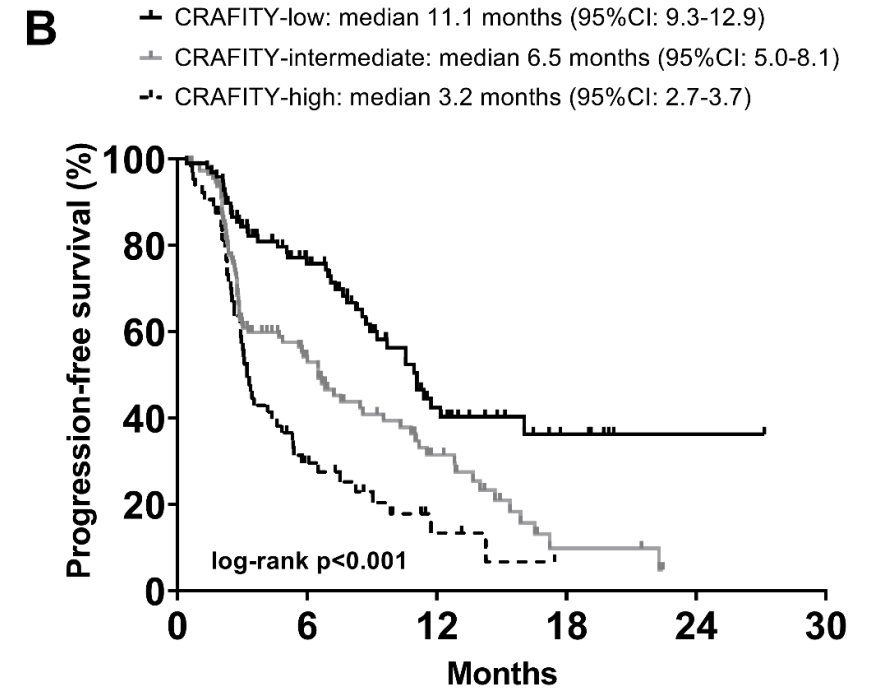
Results: Overall, 274 patients (66.1±11.0 years; male: n=224, 82%) were included, of which 208 (76%) had cirrhosis. Most patients had BCLC C (n=198, 72%). While 97 patients (35%) had CRAFITY-low, n=113 (41%) and n=64 (23%) had CRAFITY-intermediate and CRAFITY–high, respectively.
Median OS (Panel A) and PFS (Panel B) were significantly worse in patients with higher CRAFITY scores (OS: low: 23.4 (95%CI: 14.8-32.0) vs. intermediate: 15.9 (95%CI: 11.9-19.9) vs. high: 8.6 (95%CI: 5.6-11.6) months, p<0.001; PFS: low: 11.1 (95%CI: 9.3-12.9) vs. intermediate: 6.5 (95%CI: 5.0-8.1) vs. high: 3.2 (95%CI: 2.7-3.7) months, p<0.001). Upon multivariable analyses, CRAFITY was independently associated with OS (aHR: intermediate vs. low: 1.51 (95%CI: 0.92-2.48), p=0.103; high vs. low: 2.56 (95%CI: 1.52-4.33), p<0.001) as well as PFS (aHR: intermediate vs. low: 1.77 (95%CI: 1.21-2.59), p=0.003; high vs. low: 2.90 (95%CI: 1.91-4.39), p<0.001).
CRAFITY was also significantly associated with radiological response (complete/partial response (CR/PR) / stable disease (SD) / progressive disease (PD), which was evaluable in 245 patients (89%): low: n=34 (38%) / n=45 (50%) / n=11 (12%) vs. intermediate: n=37 (37%) / n=28 (28%) / n=34 (34%) vs. high: n=12 (21%) / n=18 (32%) / n=26 (46%); p<0.001). Disease control rates (DCR) were 88% vs. 66% vs. 54% (p<0.001), respectively. Upon multivariable logistic regression, a higher CRAFITY score was independently associated with a lower probability of disease control (aOR: intermediate vs. low: 0.25 (95%CI: 0.11-0.55), p=0.001; high vs. low: 0.15 (95%CI: 0.06-0.35), p<0.001).

Conclusion: The CRAFITY score identifies AB treated patients with a favourable prognosis and response and may help with patient counselling.

Figure: Comparison of overall **(A)** and progression-free survival **(B)** across CRAFITY score



No. at risk						
0 points	97	66	34	8	2	1
1 point	113	60	27	11	3	1
2 points	64	32	8	2	1	1



No. at risk						
0 points	97	56	21	7	2	1
1 point	113	46	18	4	1	1
2 points	64	16	4	1	1	1

P01-16
Validation of serum biomarker panels for early HCC detection: Results from a large prospective Latin American multicenter study

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Background and Aims: HCC is a major cause of cancer death. Guidelines recommend routine 6-month ultrasonography surveillance for high-risk patients, but its effectiveness in early-stage HCC detection is limited. PIVKA-II, AFP, and the GALAD panel of serum biomarkers are linked to HCC, but inconsistent use in guidelines limits their value.

Method: In a multi-center study, 2045 patient samples were retrospectively or prospectively collected from 7 countries in Latin America and Europe and analyzed for cancer diagnosis and liver disease etiology. The performance of multivariable models based on AFP and PIVKA were tested for early-stage HCC detection, low AFP HCC, 12 months pre-diagnostic HCC (n=92, range 9-15 months), and compared to cirrhosis and other liver tumors.

Results: The GALAD model showed excellent ability to differentiate HCC from liver cirrhosis in our prospective Latin American cohort, with an AUC of 87.9. Sub-analysis of early HCC, and stratification for etiology still demonstrated excellent performance in Latin American cohort. Moreover, a novel multivariable model was developed to detect early-stage HCC with low AFP levels, by combining sex, age, AFP, and PIVKA-II (also called GAAD), but without AFP-L3, which resulted in AUC of 87.3. Both GALAD and GAAD effectively differentiated low AFP HCC from cirrhosis in both European and Latin American patients, with AUCs of 82.8 and 81.6, respectively. Importantly, GAAD differentiated non-cirrhotic HCC (n=243) from other malignant and benign liver tumors with an AUC of 91.9, and it was 100% sensitive and specific in hemangioma cases (n=64).
Finally, we observed that even prior months to HCC diagnosis, GAAD differentiated cirrhosis (n=193) from pre-diagnostic HCC (n=92) ($p<0.0001$) in those who would develop an advanced HCC in 9-15 months, with more than 60% of patients having score above threshold.

Conclusion: We validated for the first time the GALAD model in our large cohort of Latin American HCC and cirrhosis patients. Wed demonstrated comparable performance of GALAD model with the GAAD model developed on data from our European Latin American cohorts. The models are robust in early stage HCC and diverse patient populations. Finally, very importantly, in a large cohort of 92 serum samples collected 9-15 months prior to HCC diagnosis elevated GAAD scores were observed. Our findings warrant its consideration for inclusion in international guidelines for HCC diagnosis and surveillance. With high accuracy, sensitivity, and specificity, the GALAD and GAAD model has the potential to revolutionize the routine HCC surveillance and diagnosis, in both high-risk cirrhotic and non-cirrhotic cases patients and in low AFP early stage HCC, also in Latin America.

P01-18
Prognostic performance of Toronto HCC risk index in patients with Hepatocellular carcinoma

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Background and Aims: Hepatocellular carcinoma (HCC) is a life-threatening complication of cirrhosis. The Toronto HHC risk index (Toronto index) which is a simple score recently proposed for the prediction of HCC, could have a prognostic value. Our objective was to assess the prognostic performance of the Toronto index at the time of diagnosis of HCC on the prediction of overall one-year survival.

Method: This was a retrospective study including consecutive cirrhotic patients with HCC followed in our department, between January 2010 and December 2019. Overall survival was assessed by Kaplan-Meier survival analysis using log-rank. Demographic, clinical, and paraclinical data were collected.

Results: A total of 219 cirrhotic patients were included. Sixty-one (27,8%) of them had HCC with a mean age of 64,3 ± 10,1 years and a sex ratio of 3,35. The patients were classified according to the BCLC classification: 3,2% stage (0), 33,8% stage (A), 28.9% stage (B), 19,1% stage (C) and 15 % stage (D). At a threshold of 226, the Toronto index had a sensitivity and specificity in predicting HCC of 80.3% and 48% respectively with an area under the ROC curve of 0.69 [95% CI: 0.61-0,76]. Toronto index was statistically associated with BCLC classification (p=0,011). Twenty-one patients (classified as stage BCLC 0 and A) underwent curative radiofrequency treatment (34,4%) and two patients underwent surgical resection (3,2%). Thirteen patients classified as stage B underwent chemoembolization (21,3%) and three patients were treated with sorafenib (4,9%). One-year overall survival was 83,6%. Toronto index was not statistically associated with one-year survival (p=0,136).

Conclusion: Despite its good predictive value for the development of HCC, the prognostic performance of the Toronto index is not significant.

P01-19
Ablation of large HCC using a new mini-invasive RFA device with 4 very thincool-tip needles

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Background and Aims: We Evaluated a new radiofrequency ablation (RFA) device in the treatment of large(>4cm) hepatocellular carcinoma (HCC). Aims of the study were to assess:a)efficacy of this new RFA-device. b)safety of the technique.

Method: 16 consecutive large HCC nodules ≥4 cm in diameter in 11 patients (8 with single HCC, two with 2 and one with 4 nodules) were treated with Ultrasound(US) guided percutaneous Ablation using a powerful (200 Watt) RFA-generator with four output socket for application of up to 4 very thin (18G) Cool-Tip-needles operating simultaneously during the ablation session (CoaTherm AK-F200 - APRO KOREA inc.) . All tumors were evaluated with CEUS (contrast enhanced Ultrasound) and trhee-phase enhanced CT within seven days before ablation procedure. Treatments were performed in general anesthesia. Treatment efficacy was assessed by CEUS 24 hours post procedure and three-phase contrast-enhanced computed tomography (CT) three weeks after procedure .

Results: 24-hours-post-treatment CEUS showed complete necrosis in 14/16 HCC nodules (87%) .CT showed complete necrosis in 13/16 HCC nodules (81%). 3 months follow-up did not show local recurrence in all patients. No major complication occurred. Self limiting peritoneal effusion in 4/11 (37%), pleural effusion in 6/11 (54%) patients were treated with medical therapy

Conclusion: the new RFA device seems to be a valid and safe tool for treatment of large HCC. The advantages over other systems, in our opinion, are the very thin size of electrodes and the possibility to design a variable geometry of the desired ablation area in the liver.

P02-03-YI
Management of varices but not anticoagulation is associated with improved outcome in patients with hepatocellular carcinoma and macrovascular tumour invasion

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Background and Aims: The value of bleeding prophylaxis and anticoagulation in patients with hepatocellular carcinoma (HCC) and macrovascular tumour invasion (MVI) is unclear. We evaluated the impact of anticoagulation on thrombosis progression, bleeding events, and overall mortality, and assessed the efficacy of adequate management of varices as recommended for patients with cirrhosis.

Methods: HCC patients with MVI who had Child-Turcotte-Pugh A-B7 were included between Q4/2002 and Q2/2022. Localization of the tumour thrombus and changes at 3-6 months were evaluated by two radiologists. Univariable and multivariable logistic/Cox regression analyses included time-dependent variables (i.e., anticoagulation, systemic therapy, non-selective beta blocker treatment). The occurrence of portal-hypertension-related complications was recorded.

Results: Of 124 patients included (male: n=110, 89%), MVI involved the main portal vein in 47 patients (38%), and 49 individuals (40%) had additional non-tumorous thrombus apposition. Fifty of 80 patients (63%) with available endoscopy had varices. Twenty-four individuals (19%) received therapeutic anticoagulation and 94 patients (76%) were treated with effective systemic therapies. The use of therapeutic anticoagulation did not significantly affect the course of the malignant thrombosis at 3-6 months. Systemic therapy (aHR: 0.26 [95%CI: 0.16-0.40]) but not anticoagulation was independently associated with reduced all-cause mortality. In patients with known variceal status, adequate management of varices was independently associated with reduced risk of variceal bleeding (aHR: 0.12 [95%CI: 0.02-0.71]). In the whole cohort, non-selective beta blockers were independently associated with reduced risk of variceal bleeding or death from any cause (aHR: 0.69 [95%CI: 0.50-0.96]).

Conclusion: Adequate bleeding prophylaxis and systemic anti-tumour therapy but not anticoagulation were associated with improved outcomes in patients with HCC and MVI.

P02-08
Phenotypic characteristics of primary cholangiocarcinoma in a large French cohort of patients with viral chronic liver disease followed-up before and after viral eradication: an ANRS study

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Background and Aims: Cholangiocarcinoma (CCA) is the most common biliary tract malignancy and the second most common primary liver cancer (PLC) after hepatocellular carcinoma. Viral chronic liver disease (VCLD) is known to be a risk factor for CCA. Our study aims to describe the phenotypic characteristics of CCA occurring in a large cohort of patients with VCLD, followed-up before and after viral eradication.

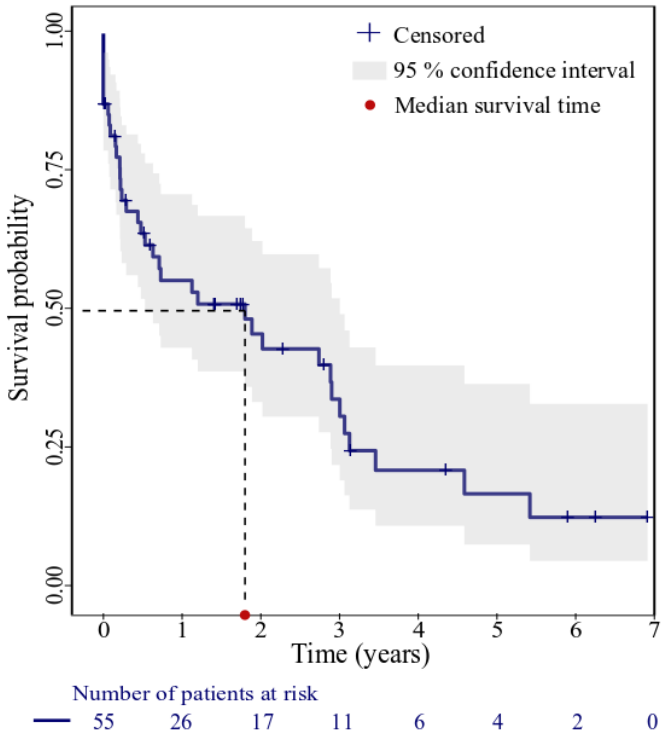
Method: 20,900 patients with VCLD included in French ANRS cohorts HEPATHER, LICAVIR and CIRVIR were analyzed. Epidemiological, radiological, histological data were recorded and assessed. Patients were followed up at least 62 months [IQR: 32-85]. Statistical analysis was performed using SAS 9.4.

Results: Among the 20,900 patients, 58 developed a histologically proven CCA. Most patients were males: 41 (71%) with median age of 62.3 years. 50 patients (86%) had hepatitis C virus (HCV) infection, 7 (12%) had hepatitis B virus (HBV) infection and 1 (2%) was coinfected. Among HCV patients, 85% had an undetectable HCV virus at CCA diagnosis. The mean time between the sustained virological response and CCA diagnosis was 36.5 months. The main cofactors for chronic liver disease were: excessive alcohol consumption (38%), arterial hypertension (36%), diabetes (22%). 55% of patients had a history of smoking. 46 patients (79%) had cirrhosis, among whom 79% had a Child-Pugh A score. Portal hypertension was present: splenomegaly in 22% of patients, ascites in 16%, esophageal or gastric varices in 27%. Most of CCA were intrahepatic (iCCA) in 27 patients (69%), 7 patients (18%) had an extrahepatic CCA (eCCA) and 5 patients (13%) had a mixed type – iCCA and eCCA. At diagnosis, 23 iCCA (72%) were single tumors, with a mean nodule size of 40.5 mm. 8 iCCA (25%) were multinodular, one iCCA was diffuse. 17 CCA (40%) were already metastatic at diagnosis. 22 CCA patients (40%) were allocated to surgical treatment, 3 patients (5%) to percutaneous tumor ablation, one patient to selective internal radiation therapy and 22 patients (40%) received chemotherapy. Most

used first line chemotherapy was gemcitabine and cisplatin. 7 patients (13%) were not eligible for any treatment at diagnosis The median survival of CCA patients was 1.8 years (Q1-Q3: 0.2-3.1 years).

Conclusion: In our large cohort of patients with VCLD, followed-up before and after viral, CCA developed mostly in cirrhotic liver. The most frequent type of CCA was intrahepatic, most of them being single tumors at diagnosis, reflecting the benefit of PLC surveillance in patients with advanced chronic liver disease. One third of CCA were already metastatic at diagnosis. Only 40% of CCA patients were eligible for a curative treatment at diagnosis. The prognosis was poor with a median survival of 1.8 years.

Figure: Median survival of cholangiocarcinoma patients.



P02-11
The importance of contrast enhanced ultrasonography and elastography as predictors for hepatocellular carcinoma recurrence following treatment with percutaneous microwave ablation or transarterial chemoembolization

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Background and Aims: Contrast-enhanced ultrasonography (CEUS) and elastography are a non-invasive means of evaluating focal liver lesions and peritumoral tissue. There is usually extensive fibrosis in the peritumoral tissue of hepatocellular carcinoma (HCC) due to the production of profibrotic mediators that could be enhanced by hypoxia in the fibrotic liver. Interventional therapies, transarterial chemoembolization (TACE) or percutaneous microwave ablation (MWA) can aggravate this hypoxia and consequently fibrosis. This study aims to evaluate the changes in liver stiffness following HCC treatment using MWA and TACE and assess whether changes in the liver stiffness are predictive factors for HCC recurrence.

Method: This is a prospective observational study including 136 HCC patients from March 2018 to March 2021, who underwent a first therapeutic procedure- either percutaneous MWA (30 patients) or TACE with doxorubicin and lipiodol or drug eluding beads (106 patients). Patients who did not have complete response on CEUS evaluation (using SonoVue) 24 hours after the procedure were excluded from further analysis. Control CT scan was performed to assess the accuracy of CEUS. In patients with complete response we performed elastography of the peritumoral tissue (2 cm margin) at one, three, six and twelve months after the procedure. We assessed HCC recurrence by CT scan (at 1 and 6 months) or MRI (at 3 and 12 months).

Results: The mean age in the study group was 54.53 +/- 17.80 years without significant difference between TACE and MWA patients. Out of the 136 patients 17 had remaining tumoral tissue after the procedure (6.66% of MWA patients and 14.15% of TACE patients). CEUS was able to assess neoplastic tissue in all patients (accuracy 100%). Patients with complete response (28 MWA patients and 91 TACE patients) were monitored by elastography (table). Recurrence occurred in 3 MWA patients (10%) and 17 TACE patients (18.68%) at one year. Patients with HCC recurrence had increased peritumoral stiffness. Also patients who underwent TACE had increased stiffness compared to MWA patients.

Conclusion: CEUS and elastography can be a reliable methods in monitoring patients after HCC treatment for disease recurrence or progression. We suggest that CEUS and elastography should be performed prior to HCC treatment as a landmark evaluation and, if nodule visualization is correct, both can be used as a follow-up alternative for more expensive imagistic methods.

Table: Comparison of liver stiffness.

Peritumoral stiffness (kPa)	MWA N=27			TACE N=91		
	Recurrence N=3	Non-recurrence N=24	P	Recurrence N=17	Non-recurrence N=74	P
1 month	12.4+/-1.17	10.3+/-1.04	0.04	22.27+/-2.04	15.26+/-1.09	0.03
3 months	16.5+/-2.8	11.07+/-2.03	0.02	24.74+/-1.65	17.36+/-2.14	0.02
6 months	18.36+/-1.27	11.3+/-1.92	<0.001	27.19+/-1.87	20.14+/-1.56	0.02
12 months	18.52+/-2.46	11.2+/-1.76	<0.001	27.35+/-2.54	21.03+/-1.26	0.02

P02-12
Updated survival outcomes with ivosidenib in patients with previously treated IDH1-mutated intrahepatic-cholangiocarcinoma: an Italian real-world experience

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Background and Aims: The results of the phase III ClarIDHy trial led to the FDA approval of ivosidenib as a therapeutic option for patients with locally advanced or metastatic cholangiocarcinoma (CCA) harboring IDH1 mutations. We recently published the first data on the use of ivosidenib in a real-world setting. Here we report the updated survival results of 11 patients with locally advanced or metastatic IDH1-mutated CCA who received ivosidenib in clinical practice.

Method: Patients treated with ivosidenib as second- and third-line treatment for advanced CCA have been collected with the aim to evaluate the survival outcomes. A molecular study has been performed by next generation sequencing essay.

Results: Overall, 11 patients were included. After a median follow up of 13.7 months, median progression-free survival from the start of treatment with ivosidenib was 4.4 months (95% CI 2.0-5.8), whereas median overall survival was 15.0 months (95% CI 6.6-15.0) regardless of treatment line. Disease control rate was 63%, with two patients achieving a partial response (18%). 18% of patients experienced at least one treatment-related adverse events (AEs), but no grade ≥ 3 were reported. The most frequently observed grade 2 AEs were prolonged QT interval and hypomagnesemia. A molecular profiling was performed on eight out of eleven patients, highlighting TP53, BAP1, CDKN2A and CDKN2B as the most common co-altered genes in these patients.

Conclusion: The present update confirms the results of our previous real-world experience on the use of ivosidenib in IDH1-mutated CCA. Real-world evidence on larger numbers of patients is needed to confirm our findings.

Figure:

Patient ID	Extent of Disease	Setting	Best Response	Progressed Disease under Ivosidenib	PFS (months)	OS (months)	*IDH1* mutation	Concomitant genetic alterations
1	M	III L	PD	Yes	3.7	8.8	R132C	*PIK3CA, TP53*
2	M	III L	PD	Yes	3.3	6.6	R132C	*BAP1, NRAS*
3	LA	III L	SD	No	11.1	11.1	R132C	*BAP1, RAD21*
4	LA	III L	SD	Yes	4.3	8.6	R132C	*ARID1A, NOTCH, NTRK1, CDKN2A/2B, BRCA1*
5	M	II L	SD	Yes	5.8	15.0	R100Q	*PBRM1, TP53, EGFR, EPHB1, AXIN1*
6	LA	II L	SD	Yes	4.4	10.2	R132C	*PSM2*
7	M	III L	PR	Yes	5.8	10.7	NA	NA
8	M	III L	PR	No	16.45	16.45	NA	NA
9	LA	IV L	SD	No	13.7	13.7	R132G	*FGFR2, BAP1*
10	LA	III L	PD	Yes	2.0	7.3	R132S	MTAP, CDKN2A/2B, KRAS, PDGFR

P02-15
Interim analysis of the ACTION trial: Cabozantinib for hepatocellular carcinoma patients who discontinued first line treatment different to sorafenib or due to sorafenib intolerance

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Background and Aims: The landscape of hepatocellular carcinoma (HCC) changed in the last 5 years. Cabozantinib was approved for HCC, but the outcome of HCC patients who received cabozantinib as second- line due to sorafenib intolerance or after discontinuing first line treatment other than sorafenib, mostly come from retrospective analysis. This clinical trial evaluates the safety profile established by the rate of adverse events (AE), rate of related-AEs and rate of death in HCC patients who received cabozantinib in second-line.

Method: Phase II, open label and investigator initiated clinical trial (CT) including HCC patients intolerant to sorafenib or those who discontinued first-line treatment with lenvatinib or atezolizumab-bevacizumab. Cabozantinib was initiated at 60 mg every day, which was modified upon development of AE. Treatment continued until symptomatic tumor progression, unacceptable AEs, patient's decision or death. An interim analysis was planned when 14 patients had a minimum follow-up of 30 days, while the CT would have to be stopped because of futility if there were 8 or more patients with critical AEs according to investigators.

Results: At November 2022, 22 patients had been enrolled: 19 included, 11 on treatment and 8 discontinued cabozantinib. Four patients discontinued due to symptomatic progression, and the other 4 due to anorectal hemorrhage, intestinal ischemia, hand-foot skin reaction grade 3 and investigator decision, respectively. Twelve out of the 14 patients with >30 days follow-up (interim analysis) were sorafenib intolerant, 6 were BCLC-C and all had preserved liver function when starting cabozantinib. Eight patients developed 17 AE > grade 3, 11 of them were cabozantinib-related and 7 meet the definition of serious adverse events (SAE). Table 1 shows the 7 SAEs observed in 5 patients, among which 4 SAEs were cabozantinib-related and occurred in 3 patients.

Conclusion: The Data and Safety Monitoring Board concluded that the ACTION trial could continue, since it did not meet the safety futility criteria. The final analysis is expected on June 2023.

P02-16
Culturally and linguistically diverse regions exhibit poor Hepatocellular screening uptake and low overall survival

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Background and aims: The incidence of HCC was recently shown to be under-reported in Australia. We aimed to determine the HCC landscape in a socio-demographically disadvantaged metropolitan catchment area of and assess HCC outcomes as compared to recently reported state-wide data.

Methods: This was a retrospective study of all adult patients being diagnosed and treated for HCC at this site between 1st June 2005 until 1st June 2020.

Results: 235 patients were eligible for inclusion. The majority of HCC was diagnosed at a later BCLC (B/C/D) class, n=156 (66.3%). Eighty-five patients (36%) were diagnosed on screening, with 67.3% (101/150) of those eligible not participating in screening. A later BCLC at diagnosis portended to higher mortality (HR 5.5, 95%CI 3.2-9.3, p<0.01) as did a diagnosis of HCC based on symptoms or biochemically (HR 0.53, 95% CI 0.34-0.81, p<0.01), age>70 at diagnosis (HR 2.1, 95%CI 1.23-3.5, p=0.006), higher Child Pugh score (B/C), and a higher AFP level (400 compared to <400kU/L).

Conclusion: In an inner metropolitan population with well-established screening practices, screening uptake and HCC outcomes were worse than what has recently reported. This study reveals the urgent need for resource allocation, and both patient and practitioner education and engagement pertaining to HCC in areas of sociodemographic disadvantage.

Table 1. Multivariate analysis Cox proportional hazards regression for mortality

Variable	Hazard Ratio	95% CI	p-value
Cirrhotic	0.59	0.18-3.68	0.48
Dx on HCC surveillance	0.52	0.34-0.81	<0.01
Sex (male)	0.88	0.55-1.47	0.62
BCLC Class at diagnosis (late)	4.51	2.55-7.88	<0.01
Age <50	0.55	0.20-1.25	0.19
AFP >400	1.80	1.10-2.95	0.02
Age >70	2.41	1.50-3.90	<0.01
CP score B (REF=A)	2.11	1.32-3.40	<0.01
CP score C (REF=A)	2.77	1.37-5.58	<0.01
Australian born	0.94	0.58-1.53	0.79

P02-17
Cystic Fibrosis may be an exclusion criteria for LI-RADS

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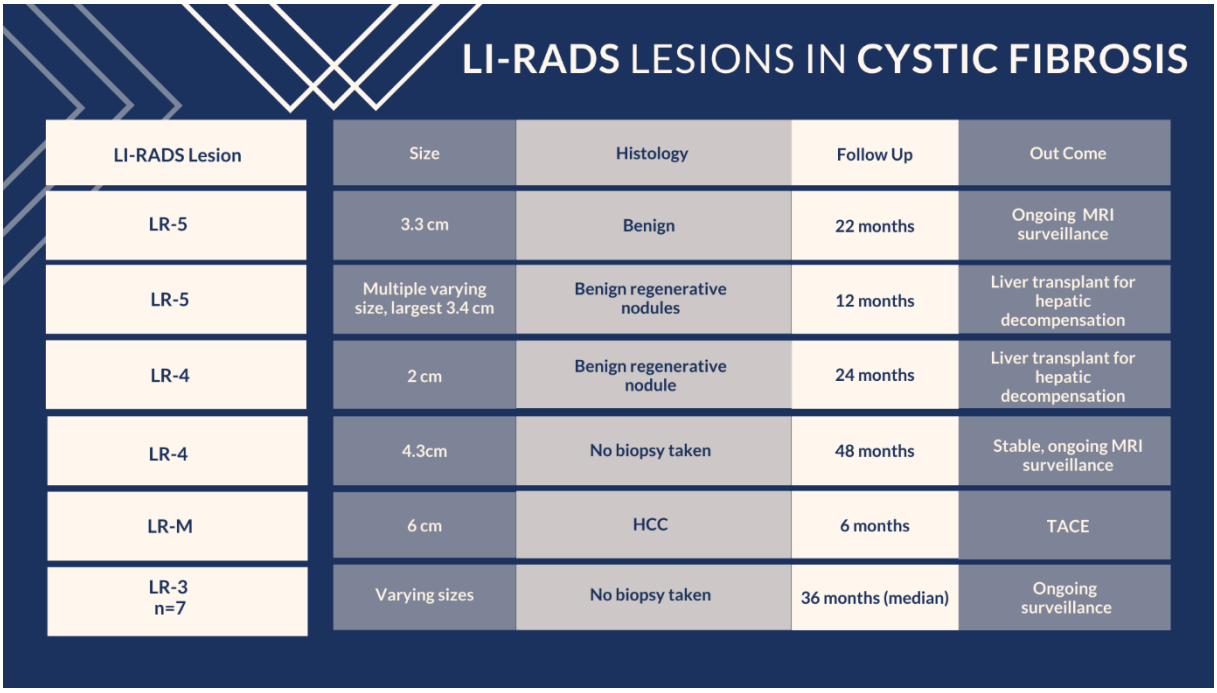
Background and Aims: Ireland has the highest prevalence of Cystic Fibrosis (CF) globally. Liver Imaging Reporting and Data System (LI-RADS) is the primary tool for diagnosing Hepatocellular carcinoma (HCC). Lesions are classified from LR-1 (definitely benign) to LR-5 (definitely HCC). The validity of LI-RADS in CF is unknown. Our aim is to identify all patients with CF who underwent liver imaging 2010-2022 with LR3-LR5 lesions and examine their outcomes.

Method: Radiology Information System was searched for “CF” and “Cystic Fibrosis”. Reports of all liver ultrasound, CT and MRI’s were reviewed. Patients with a radiologically diagnosis of cirrhosis were identified including those with a liver lesion.

Results: 93 patients had radiological evidence of cirrhosis, 10 had a lesion LR3 or higher.
Patient 1 had a single 3.3cm LR5, biopsy was benign and the lesion remains unchanged on 22 month follow up. Patient 2 had >4 LR5’s, biopsy showed regenerative nodules, confirmed on explant following transplant. Patient 3 had a 2cm LR4 lesion underwent liver transplant for hepatic decompensation, explant revealed a regenerative nodule. Patient 4 had a 4.3cm LR4 lesion which did not undergo biopsy, the lesion is unchanged on 4 year follow up. Patient 5 with a 6cm LRM underwent biopsy showing HCC. In the remaining 7 patient, LR3 lesions remain stable with median follow up of 36 months (6-168)

Conclusion: HCC is rare in CF with only 4 cases reported globally. Our data suggests that LI-RADS may not be applicable in CF. Histological confirmation should be considered.

Figure:


LI-RADS LESIONS IN CYSTIC FIBROSIS
LI-RADS Lesion
Size
Histology
Follow Up
Out Come
LR-5
3.3 cm
Benign
22 months
Ongoing MRI surveillance
LR-5
Multiple varying size, largest 3.4 cm
Benign regenerative nodules
12 months
Liver transplant for hepatic decompensation
LR-4
2 cm
Benign regenerative nodule
24 months
Liver transplant for hepatic decompensation
LR-4
4.3cm
No biopsy taken
48 months
Stable, ongoing MRI surveillance
LR-M
6 cm
HCC
6 months
TACE
LR-3 n=7
Varying sizes
No biopsy taken
36 months (median)
Ongoing surveillance

P02-18
Influence of hepatocellular carcinoma surveillance programs on cirrhotic patient's survival: A real life study

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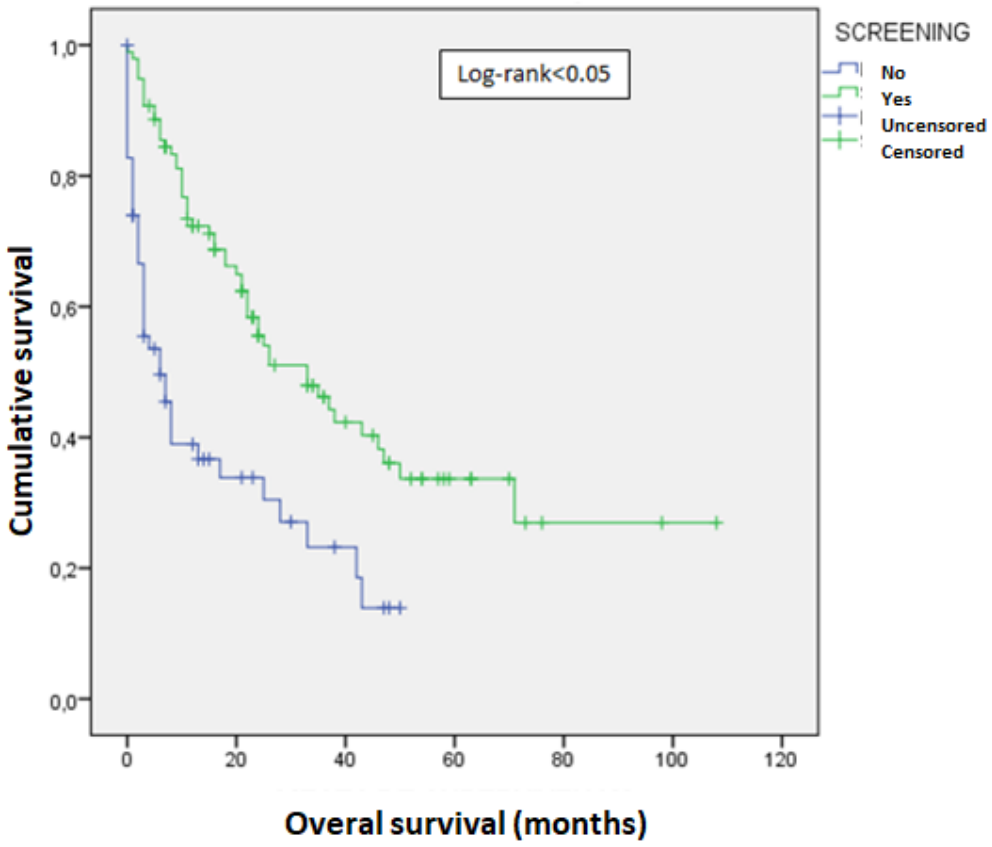
Background and Aims: Hepatocellular carcinoma (HCC) represents 75%-85% of all primary liver cancers and the third common cause of cancer-related death. Cirrhosis is the most relevant risk factor for HCC with 70%-90% of cases diagnosed at end-stage liver disease. Several studies have revealed that early diagnosis under surveillance improve prognosis and availability of major curative options. Our aim is to determinate current HCC surveillance impact on survival at a university hospital.

Method: We gathered all HCC diagnosed between Jan 2012 and May 2018 at a university hospital. Inclusion criteria was all space-occupying lesion (SOL) diagnosed at our centre and exclusion criteria were all non-HCC SOL and HCC diagnosed before or after the given dates. Epidemiological, etiological, liver-function, clinical, tumor-related, therapeutic and response-to-treatment variables were collected.

Results: The 92.5% of HCC were diagnosed on cirrhotic patients. Following the Barcelona Clinic Liver Cancer (BCLC) staging classification, 73% of patients who underwent surveillance were diagnosed on 0, A or B stages, whereas 38% of patients who didn't perform surveillance were diagnosed on given stages. The surveillance-applying cluster had a 33 months survival median while the not-surveillance cohort showed a survival of 6 months. The median survival on early stages (0 and A) [50 months], stage B (20 months), stage C (10 months) and stage D (2 months) showed statistically significant differences.

Conclusion: To undergo HCC surveillance programs allows early diagnosis on favorable tumor stages, increasing chances of major treatment options, and life expectancy of patients

Figure: Survival analysis depending on surveillance program follow-up



P02-19
Liver transplantation for HCC beyond Milan criteria after down-staging with stereotactic radiotherapy or transarterial radioembolization

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Background and Aims: Liver transplantation (LT) represents the first-line therapy for early-stage unresectable hepatocellular carcinoma (HCC). Patients (pts) with HCC outside LT-criteria may benefit from LT if successfully down-staged. According to Barcelona Clinic Liver Cancer (BCLC) staging system, transarterial chemoembolization (TACE) and systemic treatment (ST) are recommended for intermediate (stage B) and advanced HCC (stage C), respectively. We aimed at describing pre- and post-LT characteristics of pts who underwent down-staging with stereotactic radiotherapy (SABR) or transarterial radioembolization (TARE) for Milan-out HCC.

Method: We enrolled all adult pts who underwent LT in our Center from 01/01/2019 to 30/09/2022, after HCC down-staging from Milan out to Milan/Up-to-7 in criteria, by using SABR or TARE. Follow-up was closed on 31/12/2022.

Results: Among 573 LTs performed, 257 (44.9%) were due to HCC and 15/257 (5.8%) pts fit our inclusion criteria. Median age was 56 years [IQR, 54-60], 14 (93.3%) were male, mostly had HCV cirrhosis (73.3%). BMI 24 kg/m^2 [21-28], median creatinine 0.7 mg/dL [0.6-0.9], median biochemical MELD 9 [8-11]. All except 2 pts were Child-Pugh class A. Eleven pts were BCLC-B (multinodular disease) and 4 BCLC-C (portal invasion). The median number of HCC nodules was 3 [2-4] with a median size of the largest nodule of 55 mm [50-71]. Median baseline AFP value was 62 IU/mL [8-1158].
Eight pts (53.3%) underwent TARE (unilobar infiltrative HCC w/o portal trunk invasion) and 7 pts SABR (exophytic/subglissonian or hypovascularized HCC). After a median time of 8.9 [4-16] months since HCC therapy, pts were enlisted for LT (14/15 Milan-in and pt #3 Up-to-7-in). LT was performed after a median time of 23 days [8-106]. At histological examination of the explanted livers, 4 pts had no residual disease; pt #7 had cholangiocarcinoma and pt #11 had both HCC and a mixed form of cholangio-HCC. Six pts (54.4%) had poorly differentiated G3-graded HCC, and 3 pts showed microvascular invasion.As immunosuppression regimen 9 pts received everolimus plus tacrolimus (1 experienced acute rejection one month after LT) and 6 received tacrolimus plus mycophenolate. After a median follow-up of 1.8 years [1-2.6], pt #1 died for HCC metastatic recurrence 1.8 year after LT, while 14 pts (93.3%) were still alive, 12 (80%) without recurrence disease. Pt #10 was treated with TACE plus ST for HCC recurrence 1 year after LT and pt #12 underwent adrenal metastases resection 6 months after LT, both patients received pre-LT TARE for HCC with vascular invasion.

Conclusion: Our 15 HCC pts (11 BCLC-B, 4 BCLC-C) underwent LT after an overage time of 8.9 months from a successful down-staging with SABR or TARE. Fourteen out of 15 (93%) pts are still alive after a post-LT follow-up of 1.8 years; 1 pt died from HCC. 2/4 (50%) pts with baseline portal invasion showed HCC recurrence after LT.

Figure:

Patient	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15
AT DIAGNOSIS															
N° HCC	3	2	2	4	6	2	3	3	2	4	4	1	2	2	3
Max diam HCC (mm)	80	48	55	54	47	65	60	50	Infiltrative	Infiltrative	54	60	82	77	50
AFP (IU/mL)	6.3	10070	2.7	4	8.4	23.6	613	40.7	1915	40000	NA	906	83	10	756.4
Milan out	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Up-to-7 out	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y
Portal Invasion	N	N	N	N	N	N	Y, Lobar	N	Y Lobar	Y Lobar	N	Y Segmental	N	N	N
HCC THERAPY	SABR	SABR	SABR	TARE	SABR	TARE	TARE	SABR	TARE	TARE	TARE	TARE	TARE	SABR	SABR
AT LT															
N° viable HCC	1	0	2	1	2	0	0	1	0	1	1	0	1	2	3
Diam max HCC (mm)	20	0	50	20	15	0	0	40	0	14	20	0	11	22	24
AFP (IU/mL)	4.6	3.5	2.5	2.9	6.8	5.4	3.1	11.4	26.7	5.6	7.3	1.6	0.7	6	274.8
Milan out	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N
Up-to-7 out	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Portal Invasion	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
EXPLANTED LIVER															
Viable HCC	Y	N	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N	Y	Y
Differentiation grade	G3	/	G3	G3	G1	G1	G2	G2	/	G3	G3	/	/	G2	G3
Microvascular invasion	N	/	Y	N	N	N	N	N	/	Y	Y	/	/	N	N
POST LT															
Recurrence	Y	N	N	N	N	N	N	N	N	Y	N	Y	N	N	N
Death	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N

P03-04-YI
The ALBI grade refines prognostic prediction in advanced hepatocellular cancer and enables risk stratification for bleeding events following atezolizumab plus bevacizumab

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Background and Aims: The combination of atezolizumab plus bevacizumab (A+B) is the current standard of care for Child-Pugh A (CP-A) unresectable or metastatic hepatocellular carcinoma (HCC) and is being evaluated in CP-B patients. Whilst highly effective, A+B can lead to potentially life-threatening adverse events (AEs), including bleeding. We investigated whether liver dysfunction as measured by the albumin-bilirubin (ALBI) grade is associated with survival and adverse events (AEs) following A+B.

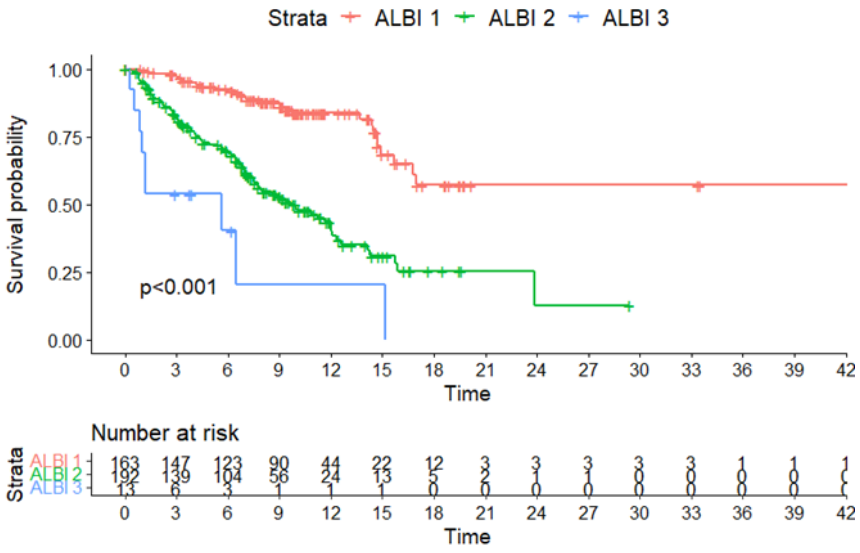
Method: We performed a multi-centre, retrospective study on patients consecutively treated with A+B in 15 tertiary referral centres. Patients exposed to systemic treatments or Child-Pugh (CP) C liver function were excluded. We correlated baseline ALBI grade with overall survival (OS) and progression-free survival (PFS) with the Kaplan-Meier method and we estimated predictors of survival with the Cox regression model. We assessed the predictive value for 6-months OS landmark with ROC curves. Association with treatment-related (tr)AEs was assessed with the chi2 test.

Results: From the initial cohort of 433 patients, 368 were included in the analysis, mostly with underlying viral hepatitis (37.5% HBV, 24.2% HCV) and a diagnosis of cirrhosis (78.8%). 295 patients (80.2%) were CP-A and 73 (19.8%) CP-B. 163 patients (44.3%) were graded as ALBI 1, 192 (52.2%) ALBI 2, and 13 (3.5%) ALBI 3. After a median follow-up of 9.7 months (95% CI, 9.2-10.3), ALBI 1 patients did not reach a median OS (mOS), ALBI 2 achieved a mOS of 9.7 months (95% CI, 6.98-12.29) compared to 5.6 months of ALBI 3 (95% CI, 0.1-12.0, p<0.001, Fig.1). Similarly, ALBI grade was associated with improved mPFS: 8.1 months (95% CI,6.0-10.2) for ALBI 1, 4.5 months (95% CI, 3.7-5.3) for ALBI 2, and 1.2 months (95% CI, 0.1-3.6) for ALBI 3 patients (p<0.001). ALBI was independently associated with OS and PFS in multivariable models (p<0.001) and outranked CP for 6-months OS prediction, with an area under the curve of 0.79 (95% CI, 0.73-0.85) for ALBI score and 0.71 (95% CI, 0.63-0.78) for CP score (p=0.013).

Whilst rates of bevacizumab-related gastrointestinal bleeding events of any grade were similar according to CP class (6.8% in CP-A vs 8.2% in CP-B, p=0.67), pre-treatment ALBI was associated with a 3-fold increase in risk of bleeding (3.1% in ALBI 1 vs 10.2% in ALBI 2/3, p=0.008). Neither ALBI grade nor CP score were associated with atezolizumab-related AEs. At treatment discontinuation (n=252, 68.5%) all patients were either ALBI 2 or 3. ALBI score predicted for worse OS after discontinuing A+B, with ALBI 2 patients achieving a post-treatment mOS of 6.8 months (95% CI, 4.4-9.2) while ALBI 3 reached 1.6 months (95% CI, 0.6-2.7, p<0.001).

Conclusion: ALBI grade identifies a subset of patients with higher probability of achieving an improved survival. Further studies should validate the role of ALBI as a predictor of bleeding events following A+B.

Figure:



P-03
Early detection of hepatocellular carcinoma via no end-repair enzymatic methylation sequencing of cell-free DNA and pre-trained neural network

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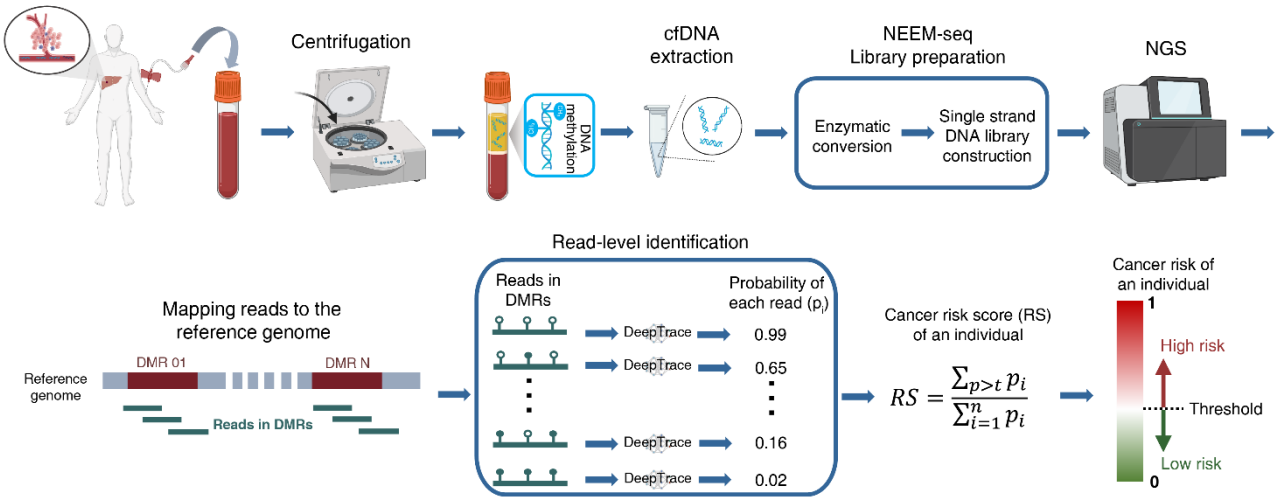
Background and Aims:
Detecting methylation aberrations in cell-free DNA (cfDNA) provides an attractive noninvasive approach for hepatocellular carcinoma (HCC) detection. Deep neural networks have been applied to genomic sequences and cancer detection recently. However, the widely-used models are difficult to learn general deep semantics of genome sequences, which may limit their performance in read-level HCC detection. In this study, we propose a read-level HCC detection model called DeepTrace to capture the genetic information of methyl-seq data.

Method:
We proposed a new methylation library construction method, low damage to DNA and high fidelity in methylation detection with No End-repair Enzymatic Methyl-seq (NEEM-seq). We further developed a read-level neural detection model called DeepTrace, which is able to better identify each HCC-derived sequencing read through a pre-trained and fine-tuned neural network. After being pre-trained on 11 million reads from NEEM-seq, DeepTrace was further fine-tuned using 1.2 million HCC-derived reads from tumor tissues DNA after noise reduction, and 2.7 million non-tumor reads from non-tumor cfDNA. The 108 individuals with cfDNA whole genome NEEM-seq data at around 1.6X depth were used to validate the model.

Results:
DeepTrace outperformed other models in identifying HCC-derived reads and in detection of HCC individuals. Based on whole genome NEEM-seq data of cfDNA, our model showed an excellent performance in detecting HCC patients with high accuracy of 97.2%, sensitivity of 95.0% and specificity of 98.5% in the validation cohort. In the early stage of HCC, the sensitivity was 93.1% for BCLC 0/A and 92.2% for TNM I, which also outperformed the Alpha Fetoprotein (AFP).

Conclusion:
By combining high-fidelity methylation data of NEEM-seq with the DeepTrace model, our method exhibits a potential for clinical application of HCC early detection with high sensitivity and specificity.

Figure:


Centrifugation
cfDNA extraction
NEEM-seq Library preparation
NGS
DNA methylation
Enzymatic conversion
Single strand DNA library construction
Read-level identification
Mapping reads to the reference genome
Reference genome
DMR 01
DMR N
Reads in DMRs
Reads in DMRs
Probability of each read (p_i)
DeepTrace
0.99
0.65
0.16
0.02
Cancer risk score (RS) of an individual
$RS = \frac{\sum_{p>t} p_i}{\sum_{i=1}^{n} p_i}$
Cancer risk of an individual
1
High risk
Threshold
Low risk
0

P03-11
Robotic and laparoscopic surgery versus open surgery for perihilar cholangiocarcinoma: systematic review and meta-analysis

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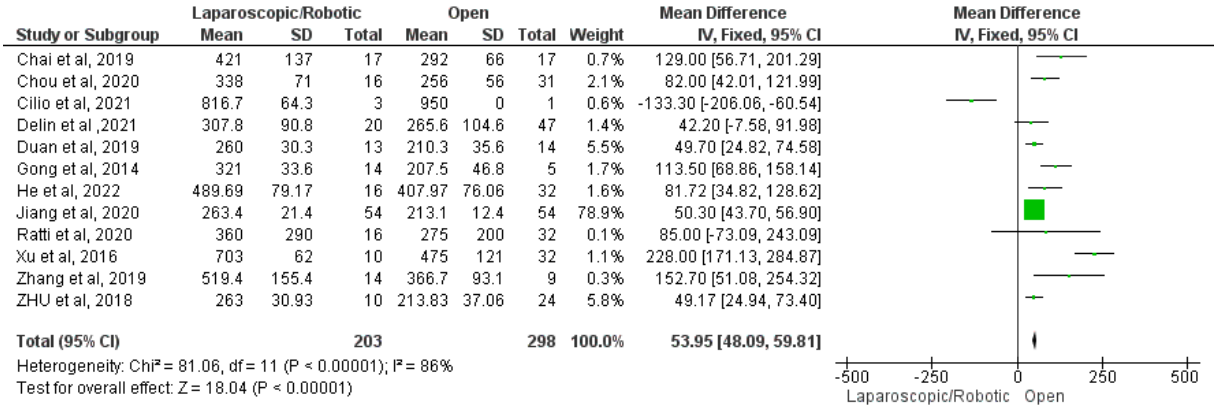
Background and Aims: Robotic and laparoscopic surgeries provide a new approach for patients with perihilar cholangiocarcinoma (pCCA). However, whether it can achieve similar outcomes to traditional open surgery (OS) remains controversial. Therefore, to compare the outcomes of laparoscopic/robotic surgery with open surgery and investigate the efficiency for Klatskin tumors further, we systematically summarized the currently available data and performed a meta-analysis.

Method: A systematic review and meta-analysis were conducted to examine the most recent studies on the topic. The search was updated to January 15, 2023, and was performed on PubMed, LILACS, and Embase. The inclusion criteria was case cohort comparing two arms: robotic or laparoscopic and open surgery for pCCA, based in PICOS principle, while the exclusion criteria were review, letter, and articles with incomplete data. All the included literature was evaluated for quality and risk of bias using the Joanna Briggs Institute's critical appraisal tool and the data were extracted and entered into an Excel spreadsheet by two authors. The data was then analyzed using Review Manager 5.4. The study protocol was registered in PROSPERO (CRD42023388478) and was conducted following the PRISMA 2020 checklist.

Results: A total of 1133 patients were included in the study (411 laparoscopic/robotic, 722 open surgeries), 15 retrospective cohort were used in the meta-analysis. The meta-analysis revealed that laparoscopic surgery resulted in less blood loss (Mean difference -77.87; 95% CI = -89.97, -67.78; p < 0,00001, I^2= 84%), shorter hospital stay (Mean difference -3.25; 95% CI = -4.83, -1.67; p < 0,0001, I^2= 79%) but longer operation time (Mean difference 53.95; 95% CI = 48.09, 59.81; p < 0,00001, I^2= 86%) (Figure 1). The mortality rate was not significantly different between the two groups (odds ratio = 0.82; 95% CI = 0.47 a 1.42; p = 0.48; I^2= 0%). Both surgeries showed similar results for age (Mean difference 1.71; 95% CI = -0.62, 4.03; p =0.15, I^2= 53%), bilirubin (Mean difference -43.45; 95% CI = -77.11, -9.79; p< 0.01, I^2= 0%), BMI (Mean difference 0.25; 95% CI = -0.24, 0.74; p= 0.31, I^2= 0%), and tumor diameter (mean difference -0.06; 95% CI = -0.52, 0.40; p =0.80, I^2= 0%).

Conclusion: The limitations of the study evidence are due to the small number of patients included in each article. Concerning blood loss and hospital stay, minimally invasive surgery offers better results. However, open surgery still has a shorter operating time. Both surgeries had similar results in mortality. Minimally invasive surgery is as safe as open surgery for pCCA resection.

Figure: Forest plot of comparison: Operation Time, outcome:



Study or Subgroup	Laparoscopic/Robotic Mean	SD	Total	Open Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95% CI
Chai et al, 2019	421	137	17	292	66	17	0.7%	129.00 [56.71, 201.29]
Chou et al, 2020	338	71	16	256	56	31	2.1%	82.00 [42.01, 121.99]
Cilio et al, 2021	816.7	64.3	3	950	0	1	0.6%	-133.30 [-206.06, -60.54]
Delin et al ,2021	307.8	90.8	20	265.6	104.6	47	1.4%	42.20 [-7.58, 91.98]
Duan et al, 2019	260	30.3	13	210.3	35.6	14	5.5%	49.70 [24.82, 74.58]
Gong et al, 2014	321	33.6	14	207.5	46.8	5	1.7%	113.50 [68.86, 158.14]
He et al, 2022	489.69	79.17	16	407.97	76.06	32	1.6%	81.72 [34.82, 128.62]
Jiang et al, 2020	263.4	21.4	54	213.1	12.4	54	78.9%	50.30 [43.70, 56.90]
Ratti et al, 2020	360	290	16	275	200	32	0.1%	85.00 [-73.09, 243.09]
Xu et al, 2016	703	62	10	475	121	32	1.1%	228.00 [171.13, 284.87]
Zhang et al, 2019	519.4	155.4	14	366.7	93.1	9	0.3%	152.70 [51.08, 254.32]
ZHU et al, 2018	263	30.93	10	213.83	37.06	24	5.8%	49.17 [24.94, 73.40]
Total (95% CI)			**203**			**298**	**100.0%**	**53.95 [48.09, 59.81]**

Heterogeneity: Chi² = 81.06, df = 11 (P < 0.00001); I² = 86%
Test for overall effect: Z = 18.04 (P < 0.00001)

P03-12
Use of cytokine TGF β1 in detection of Hepatocellular carcinoma in patients with chronic liver disease

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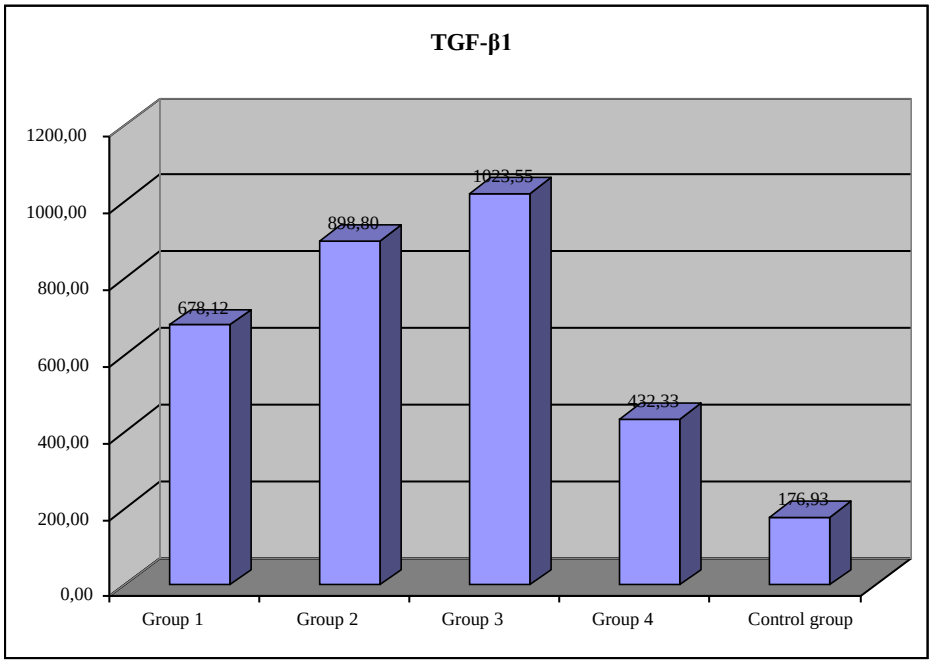
Background and Aims: The hepatocellular carcinoma (HCC) is one of the most common malignant tumor and carries a poor survival rate. Increased understanding of cancer biology and technological advances have enabled identification of a multitude of pathological,genetic, and molecular events that drive hepatocarcinogenesis leading to discovery of numerous potential biomarkers in this disease. The increased deposition of extracellular matrix proteins in the liver is a key factor in the morbidity and mortality of chronic liver disease. This increased fibrosis may be due to a superabundance of profibrogenic factors such as transforming growth factor beta. TGFβ cytokine and its isoforms initiate a signaling cascade which is closely linked to liver fibrosis, cirrhosis and subsequent progression to HCC. The analysis of in tissue levels of IL1α and TGFβ1 in HCC patients, according to BCLC scoring system has been performed, to evaluate their roles as biomarkers

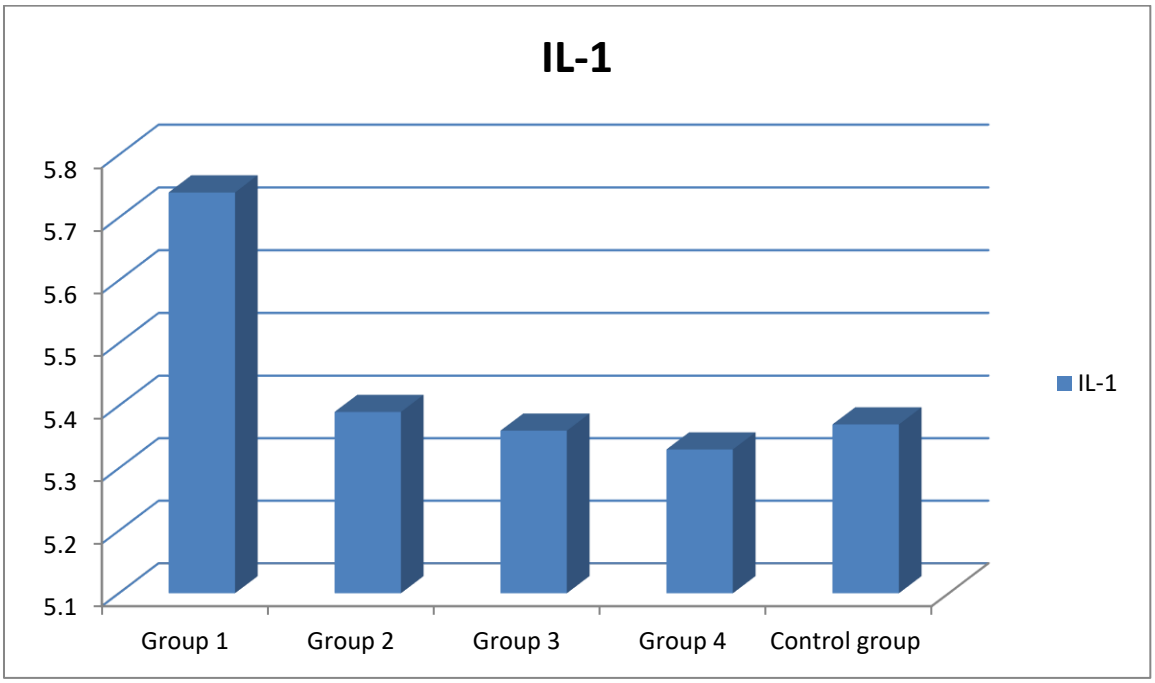
Method: Total of 150 subjects were divided into four groups, depending on the stage of HCC (BCLC). Group1 early stage; group 2 intermediate stage; group 3 advanced stage; group 4 terminal stage and the control group.The analysis included tissue levels of cytokines IL1α and TGFβ1, in bioptic matherial. FLT were also performed. Charlson comorbidity index was determined for any possible influence of other comorbid conditions.

Results: IL1 showed the highest mean concentration in group 1–early stage of HCC (5.73 pg / ml), and then in group 2 (5.39 pg / ml). IL1α was negatively correlated with the duration of the illness ($p < 0.01$) and positively correlates with serum ALT and serum AST ($p < 0.01$).The highest mean concentration of TGFβ was in group 3 (advanced stage of HCC): 1023,55 pg/ml. ANOVA analysis proves that serum levels of TGFβ1 in the control group differed with respect to the other as follows: in relation to group 1 at the level of statistical significance $p = 0.0028$; in relation to a group of 2 to $p = 0.0001$ level; in relation to group 3 at $p < 0.0001$. By Student's T test, TGFβ1 in group 1 was significantly different in comparison to group 3 at the level of significance $p < 0.0001$; groups 1vs.2 $p < 0.0001$; 1vs.4 $p = 0.003$.
Using Factor Analysis, significant stratification of predictive parameters in relation to the cytokine TGFβ1 was made:Age (negative), MELD score (negative),Child Pugh (negative), hystory of receiving transfusions,IL-1 (negative), fibrinogen.

Conclusion: Results suggests that differences in the levels of concentrations of TGFß1 can be used as biomarker for staging and monitoring the progression of HCC, as well as potential targets of therapy in stages B and C (BCLC).Serum levels of IL1α and TGFβ showed significant correlation with negative sign in HCC patients.

Figure:


TGF-β1
1200,00
1000,00
800,00
600,00
400,00
200,00
0,00
678,12
898,80
1023,55
432,33
176,93
Group 1
Group 2
Group 3
Group 4
Control group


IL-1
5.8
5.7
5.6
5.5
5.4
5.3
5.2
5.1
Group 1
Group 2
Group 3
Group 4
Control group
IL-1

P03-14
Clinical characteristics, treatments and outcomes in patients diagnosed with hepatocellular carcinoma (HCC) receiving locoregional treatment in England

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Background and Aims: The incidence of HCC in the UK has increased over the past decades. During 2013-2017, the 1-year HCC survival in England was only 46.2%. Locoregional treatment is a therapeutic option for unresectable HCC and for those awaiting liver transplant, but its use in real-world settings is not well described. This study describes clinical characteristics, treatments and outcomes in patients with HCC who receive locoregional intervention as their first treatment.

Method: This work used data collated by the National Disease Registration Service in England. Patients diagnosed with HCC (ICD10 code C220 and morphology code 8170) between 01/01/2015 - 31/12/2020, aged ≥18 years, were selected from the National Cancer Registration Dataset. A subgroup receiving transarterial chemoembolization (TACE) or transarterial embolization (TAE) or selective internal radiation therapy (SIRT)/ transarterial radioembolization (TARE) or stereotactic body radiation therapy (SBRT) as first treatment within -30 to 365 days after HCC diagnosis was selected. Follow-up ended 30/6/2022. Baseline comorbidities based on inpatient hospitalisations up to 5 years before diagnosis were identified from Hospital Episodes Statistics. Systemic treatment use was established from systemic anti-cancer therapy data. Characteristics, treatments and outcomes were described. The Kaplan-Meier method was used to estimate overall survival (OS).

Results:
Of the 3,592 patients with HCC who received locoregional treatments, 2900 patients received them as a first treatment within the study period, with TACE initiated in 65.8% (Table). Diabetes and cirrhosis were the most common conditions, 41.6% and 38.2%, respectively. Median OS from first locoregional treatment was 27.2 months (95% CI:26.1 - 28.6).

Conclusion: These real-world data improve our understanding of patients receiving locoregional treatment for HCC. Further work into subsequent treatments and clinical outcomes will highlight areas of potential unmet need.

Table:

Characteristics and treatments (N=2900)	
First locoregional treatment, n (%)	
TACE	1907 (65.8)
TAE	888 (30.6)
SBRT	92 (3.2)
TARE/SIRT	13 (0.4)
Median age at diagnosis (years)	69 (IQR:62 - 76)
Male, n (%)	2337 (80.6)
Index of multiple deprivation quintile, n (%)	
most deprived - 1	683 (23.6)
2	631 (21.8)
3	525 (18.1)
4	571 (19.7)
least deprived - 5	490 (16.9)
Comorbidities, n (%)	
cirrhosis	1107 (38.2)
alcoholic liver disease	657 (22.7)
non-alcoholic fatty liver disease	206 (7.1)
fatty liver disease	178 (6.1)
hepatitis C	8 (0.3)
hepatitis B	21 (0.7)
hepatitis E	4 (0.1)
Other chronic viral hepatitis (related to B and C)	55 (1.9)
diabetes	1207 (41.6)
oesophageal varices	727 (25.1)
kidney function impairment	391 (13.5)
gastrointestinal bleeds	296 (10.2)
portal hypertension	710 (24.5)
Median follow-up time from diagnosis (months)	26.4 (IQR:16.4 - 40.7)

IQR: Interquartile range

P03-18
Early diagnosis of hepatocellular carcinoma at the level of an infectious diseases hospital

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Background and Aims: HCC today is one of the important problems of modern hepatology, due to late diagnosis, on the one hand, and the severe course of the disease, on the other hand. In this connection, the purpose of this study was to study the effectiveness of the use of AFP and PIVKA II for the early detection of HCC among patients with liver cirrhosis at the level of an infectious diseases' hospital.

Method: 300 patients with liver cirrhosis HBV, HCV and HBV+HDV etiology were examined for tumor markers AFP and PIVKA II.

Results: Of 100 patients with HBV cirrhosis, 4 PIVKA II+AFP were above normal. The presence of formation formation in 3 patients was confirmed by ultrasound, in 1 patient the formation was not visualized by ultrasound, but was confirmed by MSCT. Of the 4 patients with an isolated increase in PIVKA II, ultrasound revealed a formation lesion in 2 patients, and a formation lesion was detected in 2 patients on MSCT. There was no isolated increase in AFP in this group. Of 100 patients with cirrhosis of HCV etiology, 6 PIVKA II+AFP were above normal. In 5 of them, the volumetric formation was visualized on ultrasound and in 1 on MSCT. An isolated increase in PIVKA II occurred in 8 patients, in 4 of them the formation was detected by ultrasound, and in 4 by MSCT. Of the 5 patients who experienced an isolated increase in AFP, only one had a volumetric formation on ultrasound. Another 2 patients underwent MSCT, which did not reveal a formation lesion, and 2 patients refused further examination. Of 100 patients with cirrhosis of HBV+HDV etiology, 5 had PIVKA II+AFP levels above normal. Of these, in 4 patients, a formation lesion was visualized on ultrasound, and in 1 patient, a formation lesion was detected on MSCT. Of the 5 patients with elevated PIVKA II levels, 3 had a formation lesion on ultrasound, and 2 patients had a formation lesion on MSCT. Of the 5 patients with an isolated increase in AFP, 1 patient had a formation lesion on ultrasound, 1 patient had no formation lesions on MSCT, and 3 patients refused further study. In patients screened at the level of the Research Institute of Virology, the average size of the tumor node was 31.59±11.68 mm.

Conclusion: The use of AFP+PIVKA II as tumor markers for the detection of HCC in patients with HBV, HCV and HDV infections makes it possible to detect HCC at an early stage and is recommended for routine use in practical healthcare, primarily at the level of infectious hospitals.

P03-20
Comparaison of prognostic scores before the first session of tranarterial chemoembolization of hepatocellular carcinoma

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Background and Aims: Transarterial chemoembolization (TACE) is offered to a heterogeneous population of patients, leading to variations in survival outcomes in many studies. Several prognostic scores, such as HAP (Hepatoma Arterial-Embolization Prognostic), NIACE (Tumor Nodularity, Infiltrative nature of the tumor, serum Alpha-fetoprotein level, Child-Pugh stage, ECOG performance status), STATE (Selection for Transarterial Chemoembolization Treatment), and Six and Twelve, have been developed to select the best candidates for TACE. The aims of our study were to evaluate the overall survival after the first TACE session for hepatocellular carcinoma (HCC) in patients with cirrhotic liver and to compare the performance of the different prognostic scores in predicting overall survival.

Method: We conducted a retrospective study including all patients followed for HCC complicating liver cirrhosis in the general surgery and gastroenterology departments of Mongi Slim Hospital who were treated with chemoembolization during the period from January 2011 to December 2020.According to the baseline characteristics, the prognostic scores based on NIACE, HAP, STATE and the Six and Twelve criteria were calculated respectively. Risk stratification and candidate identification were obtained according to previous literature. The outcome evaluation of first TACE treatment was based on overall survival (OS), which was defined as the time from first TACE to death or the end of the study. All statistical analysis were performed using SPSS software version 26.0.

Results: Fifty-five patients were included with an average age of 60 ± 12 years. The mean duration of follow-up was 16.9 months (range: 1-107). The median survival was 10 months (range: 6.4-13.26). Only the STATE and Six and Twelve scores were able to distinguish groups of patients with significantly different overall survival. The median survival in the STATE ≥ 18 group was 1 month versus 12 months in the < 18 group (p=0.008). The median survival in the Six and Twelve score ≤ 6 group was 10 months versus 8 months in the score > 6 and ≤ 12 group and 1 month in the score > 12 group. The difference was significant between the first and third groups (p<0.001) and between the second and third groups (p=0.025). No score was effective in predicting survival based on the analysis of ROC curves.

Conclusion: In our study, the STATE and Six and Twelve scores were able to select patients with the best prognosis who would benefit most from TACE. We suggest considering these two scores in multidisciplinary consultation meetings when making therapeutic decisions.

P04-02-YI
Real World Data for Atezolizumab plus Bevacizumab in unresectable Hepatocellular Carcinoma: how does the adherence to the IMbrave150 trial inclusion criteria impact prognosis?

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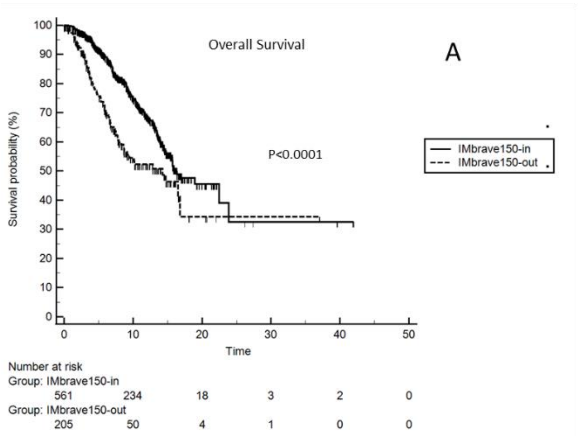
Background and Aims: Atezolizumab plus bevacizumab has been recently approved as new first-line standard of care for patients with unresectable hepatocellular carcinoma (HCC). We performed a real world study to evaluate the impact of the IMbrave150 trial inclusion criteria on safety and efficacy of treatment.

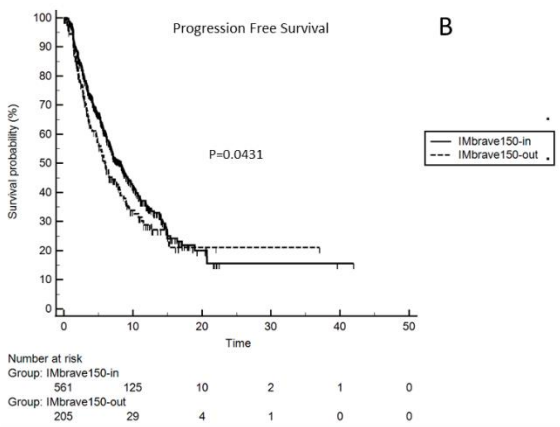
Method: We enrolled patients treated with atezolizumab plus bevacizumab for unresectable HCC from 4 different countries. No specific inclusion and exclusion criteria were applied, except for the absence of previous systemic therapies for HCC. The whole population was split in two groups according to the concordance with the inclusion criteria as reported in the IMbrave150 trial in “IMbrave150-in” and “IMbrave150-out” patients, and safety and efficacy n the two groups of patients have been evaluated.

Results: 766 patients were enrolled into the study: 561/766 (73%) were included in the “IMbrave150-in” group, and 205/766 (27%) were included in the “IMbrave150-out” group. Median OS and median PFS were 16.3 Vs 14.3 months (HR 0.48, 95% CI 0.35–0.65; p<0.0001] and 8.3 Vs 6.0 months (HR 0.79, 0.63–0.99; p=0.0431) in “IMbrave150-in” and “IMbrave150-out” patients, respectively. Multivariate analysis confirmed that patients included in the “IMbrave150-in” group had significantly longer OS compared to patients included in the “IMbrave150-out” group (HR 0.76, 0.47-0.97; p=0.0195). In “IMbrave150-in” patients the ALBI grade was not associated to OS, whereas in “IMbrave150-out” patients, those with ALBI grade 1 reported a significant benefit in terms of OS compared to those with ALBI grade 2 (16.7 Vs 5.9 months HR 4.40,2.40-8.08, p>0.0001). No statistically differences were reported in “IMbrave150-in” and “IMbrave150-out” groups in terms of safety profile.

Conclusion: The adherence to the IMbrave150 trial inclusion criteria favorably impacts the prognosis of patients receiving atezolizumab plus bevacizumab. Among patients who do not meet the IMbrave150 inclusion criteria, those with ALBI grade 1 could benefit from the treatment.

Figure:


Overall Survival
A
P<0.0001
IMbrave150-in
IMbrave150-out
Survival probability (%)
Time
Number at risk
Group: IMbrave150-in
561 234 18 3 2 0
Group: IMbrave150-out
205 50 4 1 0 0


Progression Free Survival
B
P=0.0431
IMbrave150-in
IMbrave150-out
Survival probability (%)
Time
Number at risk
Group: IMbrave150-in
561 125 10 2 1 0
Group: IMbrave150-out
205 29 4 1 0 0

P04-04
Artificial intelligence and deep learning models used for the classification and navigation of liver ultrasound protocols for patients with hepatocellular carcinoma

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Background and Aims: Deep learning methods have been used in medical imaging to aid in classification systems such as navigation systems for liver segmentation, feature extraction, and disease classification, and also to generate realistic medical images that could be used for training neural networks. Convolutional Neural Network CNN and two-phased Generative Adversarial Network GAN architectures have been used for the synthesis of images for improved classification of liver disease. In this project, we use artificial intelligence AI and deep learning models DLM to classify and navigation of liver ultrasound protocols for patients with hepatocellular carcinoma.

Method: Using AI and DLM, we build a latent diffusion model based on the Cancer Imaging Archive (TCIA) liver ultrasound dataset (Eisenbrey, J., Lyshchik, A., & Wessner, C. (2021). Ultrasound data of a variety of liver masses [Data set]) containing 197,000 images from 120 patients with different stages of liver disease and hepatocellular carcinoma HCC, doing further data augmentation using Roboflow for manually selecting regions of interest ROI using stable diffusion models.

Results: Our model can produce realistic, high-resolution images of liver ultrasounds conditioned on low-resolution semantic maps. We demonstrate the value of this model by using its outputs to train a deep neural network to identify liver ultrasounds containing steatosis, cirrhosis, and HCC. In addition, we show that a classifier trained on a mixture of real and synthetic steatosis ultrasounds outperforms models trained on real images only.

Conclusion: These findings indicate that ultrasound technicians, clinical imaging healthcare professionals, and diagnostic medical sonographers could be helped by artificial intelligence / deep learning models in their liver protocol navigation and selection of ROI, and as a reliable factor for grading fatty liver disease by using the liver-kidney density ratio.

Figure:

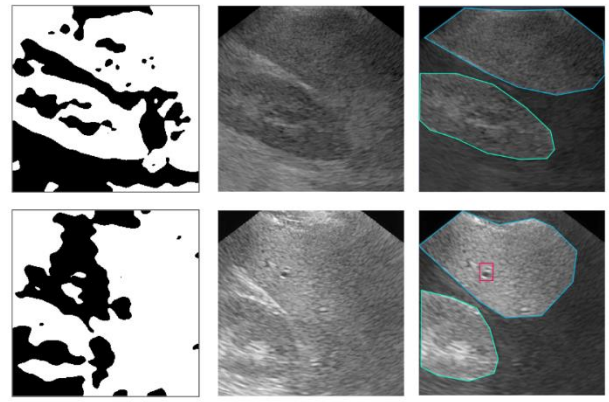

Figure 1: Qualitative examples of synthetic liver ultrasounds (middle) conditioned on semantic maps (left) and annotations by ROI (right)

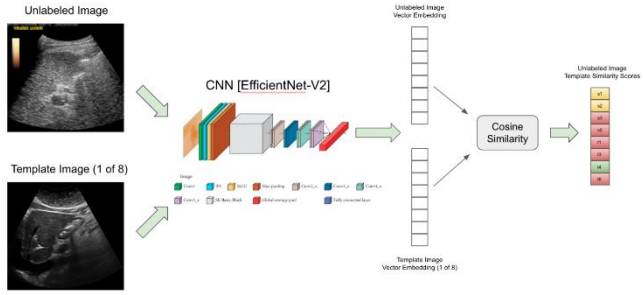

Fig 2. Auto-labeling ultrasound views (5 sagittal and 5 transverse) in liver ultrasound protocol.

P04-05
Curative-dose radiotherapy and multiagent chemotherapy improves survival of locally advanced intrahepatic cholangiocarcinoma: A retrospective multi-institutional study (KROG 20-02)

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Background and Aims: Locally advanced unresectable intrahepatic cholangiocarcinoma shows detrimental oncologic outcome. In this study, we investigated the efficacy of chemoradiotherapy in patients with locally advanced intrahepatic cholangiocarcinoma (ICC) and identify the prognostic factors for local control (LC) and overall survival (OS).

Method: We retrospectively reviewed the records of 114 patients who received radiotherapy+/-chemotherapy for ICC between 2001 and 2021. The median equivalent RT dose (EQD2) was 52 Gy. Multiagent chemotherapy was administered before or after radiotherapy in 59 patients. LC and OS were analyzed using the Kaplan–Meier method; prognostic factors were analyzed using the Cox proportional hazards model.

Results: Overall, 1-year LC and OS were 74% and 63.8%, respectively. Multivariate analysis revealed the EQD2 ≥ 60 Gy and multiagent chemotherapy as significant positive factors for LC and OS. Based on the identified risk factors, patients were grouped; EQD2 ≥ 60 Gy with multiagent chemotherapy (Group A, n=23), EQD2 < 60 Gy with multiagent chemotherapy or radiotherapy with single agent chemotherapy (Group B, n=69), radiotherapy alone (Group C, n=22). The group A had significantly better OS than the group B and C ($p < 0.05$).

Conclusion: Curative-dose radiotherapy and multiagent chemotherapy improved oncologic outcome of patients with locally advanced ICC. Further prospective study is warranted for validation.

P04-07
Portal-hypertension parameters are associated with survival and ascites occurrence in patients with hepatocellular carcinoma treated by external radiotherapy

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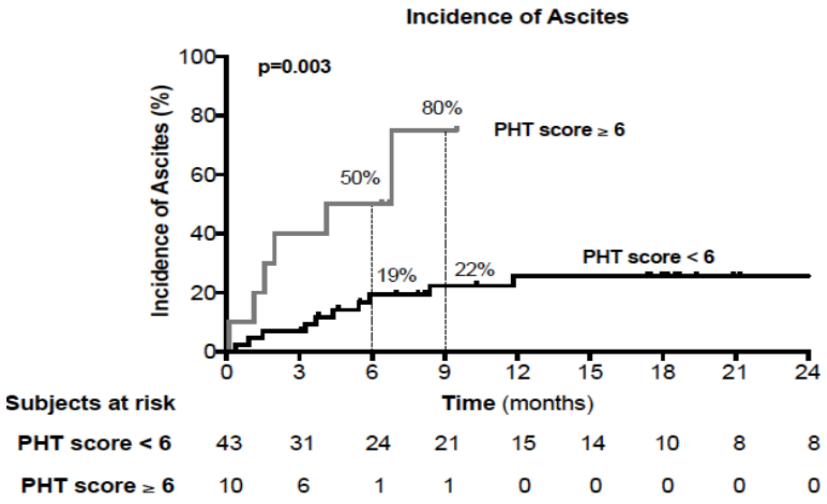
Background and Aims: Radiotherapy (RT) is part of the new HCC therapeutic arsenal but can expose to radiation induced liver disease that includes ascites occurrence. We aimed (i) to study the impact of portal hypertension (PHT) on RT outcome and (ii) to identify predictive factors of ascites occurrence.

Method: Our retrospective monocentric study included all cirrhotic patients who received either stereotactic ablation body radiation therapy (SABR) or conventionally fractionated radiotherapy (CFRT) for HCC between 2012 and 2022. PHT was assessed on upper endoscopy and on imaging using the PHT Score based on the presence on imaging of ascites, varices, and spleen size. Threshold of 6 was chosen according AUROC analyze. Time-to-events data were estimated by Kaplan-Meier with log-rank test, along with Cox-models.

Results: 60 patients were included (female 27%, median age 49 yrs, Child-Pugh A 82%, cause of liver diseases alcohol/metabolic syndrome/hepatitis C in 56/40/32%). 40% and 15% presented history of ascites and acute variceal bleeding (AVB) respectively, 26% had large esophageal varices (EV), median HTP score was 4 and 19% presented a HTP score ≥ 6. All patients underwent appropriate prophylaxis for AVB when indicated. 92% were BCLC-0/A, median tumor size was 30mm. An infiltrative form and vascular invasion were present in 2% and 3% respectively.
SABR was performed in 39 (48Gy / 9 fractions) and CFTR in 21 patients (54Gy / 29 fractions). The max PTV1 EQD2 (a/b = 10) and PTV1 volumes were 124Gy and 103cc (SABR) and 64Gy ($p < 0.001$) and 173cc ($p = 0.91$) (CFTR). Median EQD2 to uninvolved liver (a/b = 3) were 11Gy (SABR) and 13Gy ($p = 0.41$) (CFTR). Overall survival (OS) was 75%, 50% (SABR) and recurrence free survival 54%, 22% (CFTRE) at 1 and 3 years after RT. At 6 months, progressive disease according to RECIST1.1 was more frequent in patients treated by CFTR vs SABR (18% vs 7%, $p < 0.001$). In univariate but not in multivariate analysis, SABR (HR = 2.65, $p = 0.03$), albumin (HR = 1.00, $p = 0.05$), maximal PTV1 EQD2 (a/b = 10) (HR = 1.02, $p = 0.01$) and PTV1 volume (HR = 0.99, $p = 0.04$) were associated with mortality. In log rank analysis, HTP score ≥ 6 was associated with lower survival ($p = 0.07$). EV and platelets were not associated with OS.
Ascites incidence after RT was 24%, 34%, and 38% at 6, 12, and 24 months, respectively. In univariate analysis, platelets count (HR = 1, $p = 0.08$), history of AVB (HR = 2.58, $p = 0.09$) and SABR tended (HR = 2.59, $p = 0.09$) to be associated with ascites but the only significant predictive factor was a HTP score ≥ 6 (HR = 4.25, $p = 0.007$) and this result was confirmed in log rank analysis ($p = 0.003$). Large EV was not associated with ascites occurrence ($p = 0.12$). Among 14 patients who developed ascites, 11 were treated with NSBBs.

Conclusion: This retrospective study suggests that RT should be avoided in patients with a HTP score ≥ 6 to prevent ascites occurrence that could preclude access to further HCC treatments.

Figure:


Incidence of Ascites
p=0.003
Incidence of Ascites (%)
100
80
60
40
20
0
80%
PHT score ≥ 6
50%
19%
22%
PHT score < 6
0 3 6 9 12 15 18 21 24
Time (months)
Subjects at risk
PHT score < 6 43 31 24 21 15 14 10 8 8
PHT score ≥ 6 10 6 1 1 0 0 0 0 0

P04-10
Predictive performance of HCC risk scores in chronic hepatitis C patients with advanced fibrosis after achieving SVR: Real-world experience from a tertiary UK centre

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Background and Aims: Current guidelines recommend biannual hepatocellular carcinoma (HCC) surveillance in patients with advanced liver fibrosis. However, this 'one-size-fits-all' strategy places a huge burden on health care systems and regression of fibrosis may occur with disease-modifying treatments. Many models have been developed to improve risk stratification and identify low-risk populations that no longer require cancer screening. Our aim is to compare the discriminative ability of these scores in patients with chronic hepatitis C infection who have achieved sustained virological response (SVR).

Method: In this single centre, retrospective study, we compared the predictive performance of the aMAP, THRI and GES scores in a cohort of CHC with a history of advanced liver fibrosis (F3-F4). All individuals managed at King's College Hospital and achieved SVR with direct acting antivirals (DAAs) between 2014 and 2020. Follow-up data was collected until HCC occurrence, death or last follow-up visit. Cumulative incidences were calculated using the Kaplan-Meier method and compared using the log-rank test. The performance of each model was assessed using Harrell's c statistics.

Results: 367 patients were included in the analysis (median age 53.0 years, males 68.5%) and the majority had documented F4 fibrosis (64.8%). By applying the GES score, 185 (50.4%), 123 (33.5%), and 59 (16.1%) of the studied patients were at low, intermediate, and high risk for HCC. According to cumulative HCC risk, patients in the three categories showed: low-risk cohort (cumulative incidence 1.381%, 95% CI 0.749-2.348), intermediate-risk cohort (CI 2.716%, 95% CI 1.578-4.379), and high-risk cohort (6.328%, 95% CI 3.746-10.06). For the aMAP score, 81 (22.0%), 176 (48.0%), and 110 (30.0%) of the studied patients were at low, intermediate, and high risk for HCC. The 7-year HCC incidence was 0.833% (95% CI 0.212-2.267) in their low-risk group, 1.141% (95% CI 0.580-2.034) in their intermediate-risk group, and 6.366% (95% CI 4.374-8.973) in their high-risk group. Finally, by using the THRI score, 95 (25.9%), 245 (66.8%), and 27 (7.4%) of the studied patients were at low, intermediate, and high risk for HCC the cumulative incidence was 0.665% (95% CI 0.169-1.810), 2.403% (95% CI 1.629-3.427) and 12.07% (95% CI 6.715-20.130) in low, intermediate and high-risk cohorts, respectively. The Harrell's c-statistic were 0.763 (GES), 0.708 (aMAP) and 0.663 (THRI).

Conclusion: All three scores stratified our patients into low, intermediate, and high-risk groups, with very low 7-year cumulative incidence rates in the low-risk group. The GES score classified approximately half of the patients as low risk, while the aMAP and THRI classified 30% of the patients as low risk. Screening can be safely avoided in this subgroup of patients, potentially improving the cost-effectiveness of our HCC surveillance programmes.

Figure:

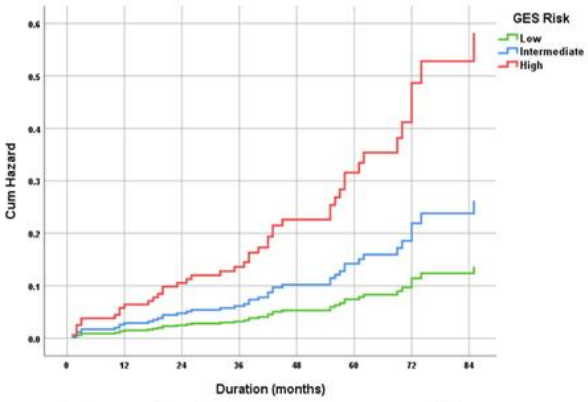

Fig. (1): Cumulative risk of HCC according to GES prediction scores

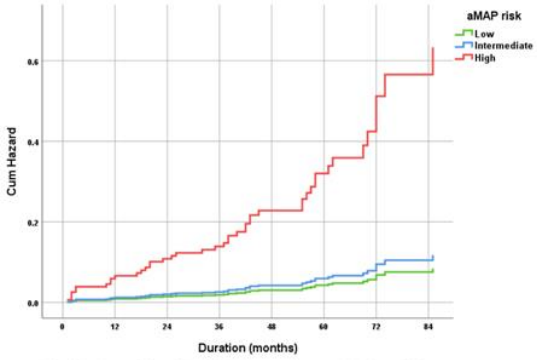

Fig. (2): Cumulative risk of HCC according to aMAP prediction scores

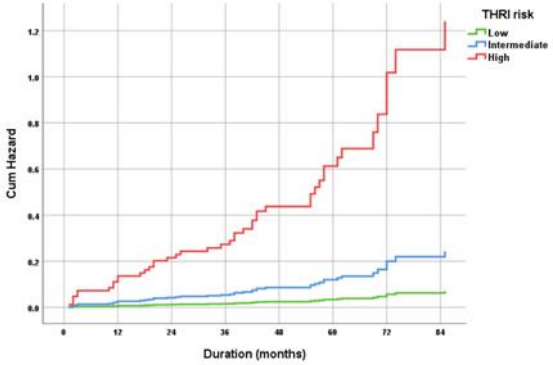

Fig. (3): Cumulative risk of HCC according to THRI prediction scores

P04-11
Hypofractionated Carbon Ion Radiation in patients with primary liver cancer

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Background and Aims: Liver cancer is the third leading cause of cancer related death due to treatment resistance and late onset of symptoms[1]. The role of external beam radiotherapy (EBRT) in treatment of unresectable liver cancer is yet to be defined. In recent years, safety and efficacy of EBRT have improved dramatically. The use of particle therapies such as carbon ion radiation therapy (CIRT) with high linear energy transfer (LET) could increase the efficacy while limiting the effects of radiation on non-cancerous liver tissue. Promising effects of CIRT have been described in several studies during the past decades, mostly in Japan and China. To date, no standardized treatment protocol has been established and European data on CIRT for liver cancer is lacking. This retrospective analysis aims to investigate efficacy and safety of hypofractionated CIRT in primary liver cancer.

Method: Thirty-six (n = 36) patients with primary malignant liver tumors (Hepatocellular Carcinoma n=32, Cholangiocarcinoma n=3, Mixed n=1) were treated with hypofractionated CIRT between 2011 and 2022 and retrospectively analyzed concerning survival, local control and toxicity. The median total applied dose was 38 Gy (Range: 35.2 – 42 Gy), divided into 4 single doses every other day.

Results: The median duration of patient follow-up was 29 months. Median size of irradiated lesions was 3.5cm (Range: 1.3 – 9.6 cm) Fifty percent of the patients had prior therapy of which 50% received transarterial chemoembolization (TACE). Only two patients experienced local progression of the irradiated lesion, resulting in a 2-year local control rate of 92.3%. The median progression free survival was 38 months. Overall Local Treatment Response Rate (PR, CR) was 64.8%. No dose limiting toxicities were reported. Adverse events (AE) of any grade occurred in 56% of all patients; the most common was fatigue (38%) followed by thrombocytopenia (14%), nausea and abdominal pain (10% each). Only two > grade 2 AEs occurred (one case of grade 3 anemia or thrombocytopenia, respectively).

Conclusion: Hypofractionated CIRT is a safe and effective treatment option for patients with liver cancer. Due to its low integral radiation dose to the remaining liver tissue it could widen the therapeutic window of liver irradiation for patients with severely impaired liver function.

1 Rumgay, H. *et al.* Global burden of primary liver cancer in 2020 and predictions to 2040. *J Hepatol* **77**, 1598-1606, doi:10.1016/j.jhep.2022.08.021 (2022).

P04-16
Serum HCC biomarkers AFP, PIVKAII and GPC-3 correlate differently with the HCC characteristics and clinic-pathologic features

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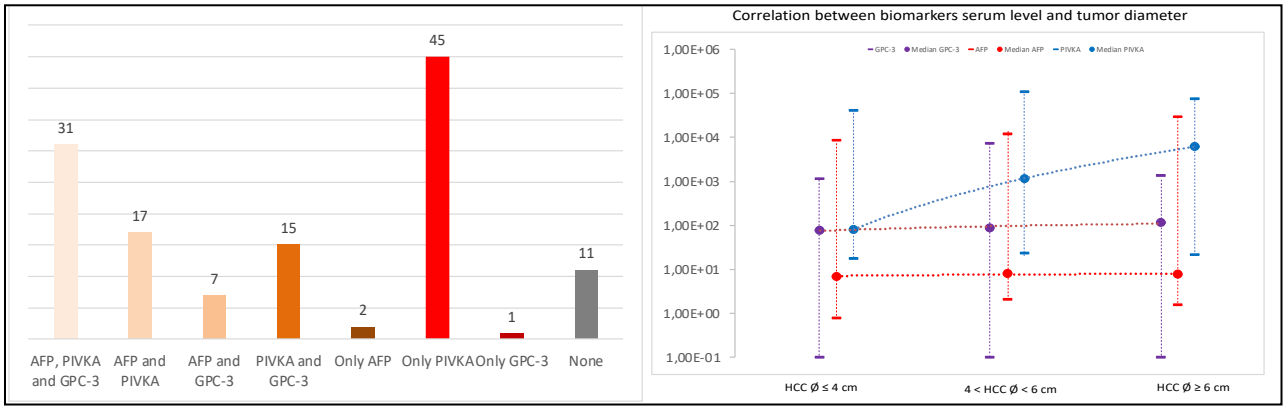
Background and Aims: The role of serum biomarkers of hepatocellular carcinoma (HCC) in the clinical management is debated. We studied the correlations of alpha-fetoprotein (AFP), protein induced by vitamin K absence-II (PIVKA-II) and glypican-3 (GPC-3) with tumor characteristics and clinical-pathological features.

Method: Since January-2015 to December-2020, 245 consecutive HCC were newly admitted at the Hepatology Unit of the Pisa University-Hospital; 129 patients (pts) [median age 70.4 years (range:48.7-88.4); M/F 94/35] fulfilled inclusion criteria: serum-samples and CT/MRI at the first observation and diagnosis, anti-HIV-negative without other neoplastic diseases. AFP, PIVKA-II and GPC-3 were measured by commercial assays (Abbott, Fujirebio); cut-off values were 10-ng/ml, 40-mAU/ml and 100-pg/ml for AFP, PIVKA and GPC-3 respectively. HCC volume was measured by CT (GE-Advantage-Workstation 4.6). Statistics used: X^2, Mann-Whitney/Kruskal-Wallis and Spearman tests and multiple regression analysis.

Results: Child-Pugh A, B, C stages of liver disease were 117(90.6%), 11(8.5%) and 1(0.9%), respectively; etiology was HBV: 21(16%), HBV+HDV: 1(1%), HCV: 55(43%), NAFLD: 35(27%) and others: 3(2%). HCC-BCLC was 0: 6(4.7%), A: 45(34.9%), B: 40(31%), C: 32(24.7%) and D: 6 (4.7%). HCC was single in 51(39.5%) and multifocal in 78(60.5%) pts; 17(13.2%) had portal-vein-thrombosis (PVT). Median volume of the largest lesion was 28.71cm^3 (0.52-1149.7cm^3). At diagnosis 118(91.5%) pts were positive for at least one biomarker: PIVKA-II in 108(83.7%), AFP in 57(44.2%) and GPC-3 in 54(41.9%). PIVKA-II was the only positive in 45(34.9%) pts, AFP in 2(1.4%) and GPC-3 in 1(0.7%) (Figure 1a). AFP and GPC-3 levels were higher ($p<0,027$ and 0,032 respectively) in females. While positivity for all markers was independent from number/size of the lesions, PIVKA-II levels correlated significantly with size (Figure 1b). AFP and GPC-3 levels were significantly higher in pts with PVT ($p=0.004$ and $p=0.002$ respectively), correlated negatively with the overall survival ($p=0.004$ and $p=0.015$ respectively) and with HCC-BCLC classes ($p<0.001$) more significantly than PIVKA ($p=0.025$).

Conclusion: At least one HCC marker was present in 91.5% of the patients at the time of diagnosis, PIVKAII being the most prevalent biomarker and correlating with tumor size. AFP and GPC-3 are less prevalent, but associated with more advanced and severe disease, PVT, and shorter survival.

Figure:


45
31
17
15
11
7
2
1
AFP, PIVKA and GPC-3
AFP and PIVKA
AFP and GPC-3
PIVKA and GPC-3
Only AFP
Only PIVKA
Only GPC-3
None
Correlation between biomarkers serum level and tumor diameter
1,00E+06
1,00E+05
1,00E+04
1,00E+03
1,00E+02
1,00E+01
1,00E+00
1,00E-01
HCC Ø ≤ 4 cm
4 < HCC Ø < 6 cm
HCC Ø ≥ 6 cm

P04-17
Multidisciplinary approach to Hepatocellular Carcinoma management in a low-income country

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Background and Aims: Hepatocellular carcinoma (HCC) management is challenging due to its complex association with cirrhosis. Though international guidelines recommend multidisciplinary approach to managing HCC patients, it is still a novel idea in low-income countries. This study gives an insight into patients managed by multidisciplinary approach.

Method: 100 patients seen from January to December 2022 were evaluated, who visited multidisciplinary HCC clinic at Aga Khan University Hospital, Karachi, Pakistan, comprising of a hepatologist, surgeon, oncologist and interventional radiologist.

Results: Mean age was 60.92 years. 72% were males, 28% were females. HCV-related chronic liver disease(CLD) was found in 60% patients,19% had Non-B Non-C CLD, 9% had HBV, 8% had HBV+HDV-CLD, 2% alcoholic CLD and 2% did not have cirrhosis. 45% patients had an ECOG score of 0, 38% were ECOG 1, 16% ECOG 2 and 1% ECOG 3. Patients with Child-class A were 65%, 27% and 6% were child class B and C respectively. LI-RAD based imaging identified 74% patients with LR-5, 15% patients with LR-4 and 8% and 1% patients had LR-3 and LR-2 lesions respectively. As per Barcelona staging system, 7% patients were stage 0, 28% stage A, 23% stage B, 26% stage C and 7% stage D. 9% patients were advised biopsy of lesion to achieve a definite diagnosis. Resection was offered to 13%, ablation to 7%. 11% were advised transplant and 2% were advised Trans-Arterial Radio-Embolization (TARE). Trans-Arterial-Chemo-Embolization(TACE) was recommended to 20% for palliation and to 2% for down staging.17% were advised chemotherapy and 7% were referred for best supportive care. Of these 100 patients, 77 belonged to Karachi while the other 33 came from outside city.

Conclusion: This study highlights the importance of multidisciplinary care in HCC management, as it provides collaborative yet individualized treatment plan to patients in a single clinical setting.

Figure:

Patient characteristics	Frequency (%)
1. Gender	
- Male	72
- Female	28
2. Etiology	
- HCV-CLD	60
- NBNC-CLD	19
- HBV-CLD	9
- HBV+HDV CLD	8
- Alcoholic CLD	2
- No underlying CLD	2
3. ECOG* score	
- 0	45
- 1	38
- 2	16
- 3	1
4. CT findings (LIRAD)	
- LR 5	74
- LR 4	15
- LR 3	8
- LR 2	1
5. CTP** Score	
- A	65
- B	27
- C	6
6. BCLC*** Stage	
- 0	7
- A	28
- B	23
- C	26
- D	7
7. Treatment recommended	
- Follow-up with imaging	9
- Resection	13
- Transplant	11
- Ablation	7
- TACE (for palliation)	20
- TACE (for down staging)	5
- TARE	2
- Chemotherapy	17
- Best supportive care	7

*ECOG – Eastern Cooperative Oncology Group
**CTP – Child Turcotte Pugh
*** BCLC – Barcelona Clinic Liver Cancer

P04-18
Serum protein glycomics in treatment-naive HCC patients are strongly associated to two-year overall survival and HCC relapse

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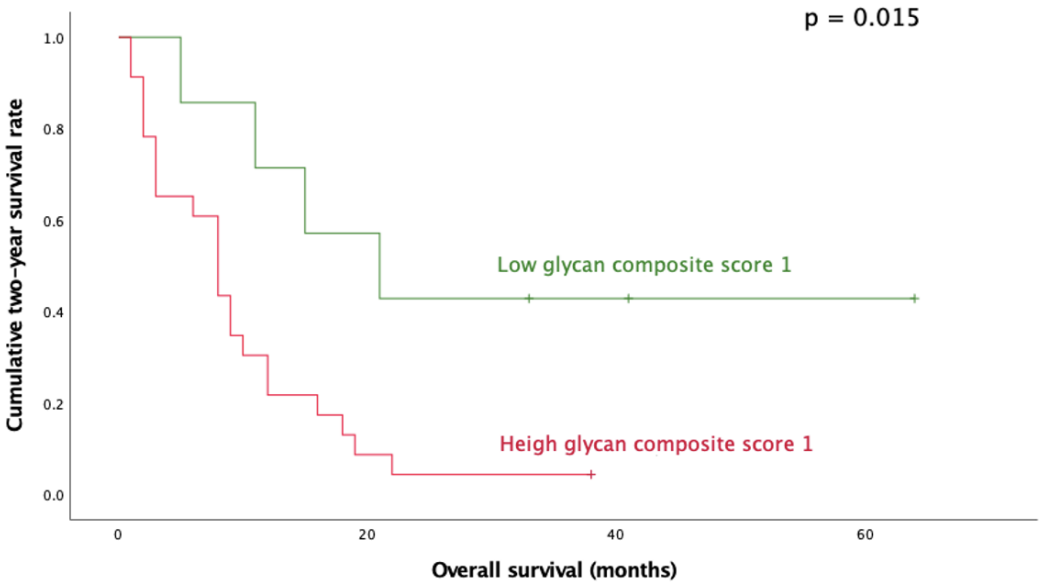
Background and Aims: Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer and is the second leading cause of cancer-related deaths globally. The currently accepted treatment options have a limited impact on survival rates. The importance of proper estimation of prognosis and effective disease monitoring is undeniable. Glycomics might be a tool to answer this medical need, since consistent modified glycosylation patterns in human proteins have been observed in HCC. The aim of this study was to examine whether specific glycomic changes in serum proteins predict outcome in treatment-naive HCC patients.

Method: A total of 46 treatment-naive HCC patients were enrolled with a serum sample at time of diagnosis. N-glycosylation patterns were analyzed according to the optimized 96-well on-membrane deglycosylation method (DSA-FACE technique). Glycan-based outcome predicters were considered using univariate log regression analysis and linked to survival rates using the Kaplan-Meier method.

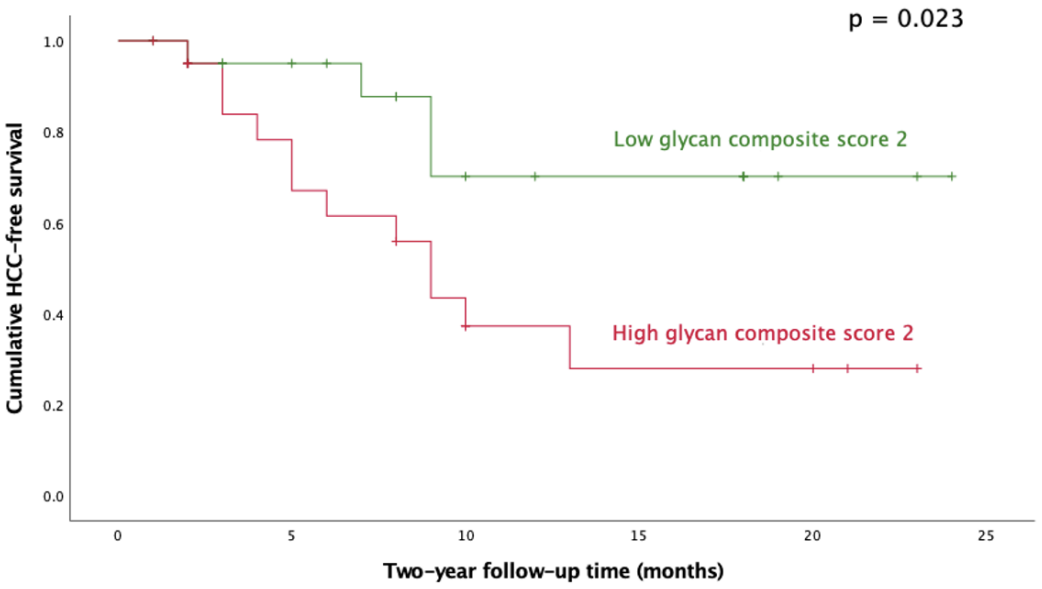
Results: The overall cohort consisted of more good responders (n = 32), according to RECIST 1.1 criteria, than poor responders (n = 14). No glycans were found to be significantly predictive of response to therapy (good versus poor responder). In contrast, upregulation of NA3Fc and NA3Fbc (glycan composite score 1, OR 2.456 [95% CI 1.304, 4.624]; P = 0.005) were predictive of poor two-year survival rate, independently of disease stage. Additionally, upregulation of NGA2F and NGA2Fb and downregulation of NA2 and NA2F (glycan composite score 2, OR 1.8 [95% CI 1.203, 2.694]; P = 0.004) were predictive of HCC relapse within two years after diagnosis. The area under the curve (AUC) of glycan composite score 1 in relation to the two-year survival rate was 0.881 ([95% CI 0.767, 0.997], Youden index = -7.0328, sens = 91.7%, spec = 84.6 %, P < 0.001), whereas the AUC of glycan composite score 2 in relation to HCC relapse was 0.813 ([95% CI 0.681, 0.944], Youden index = 6.7901, sens = 75.0%%, spec = 65.4 %, P = 0.001). The Kaplan-Meier analysis revealed that the cumulative two-year survival rate was significantly lower in patients with a glycan composite score 1 above the Youden index cut-off value (Log Rank: p = 0.015), and the cumulative incidence of HCC relapse within two years after diagnosis was significantly higher in patients with a glycan composite score 2 above the Youden index cut-off value (Log Rank: p = 0.023).

Conclusion: In this study, we demonstrated that pre-treatment tri-antennary core (and branch) fucosylated glycans are strongly associated to the two-year overall survival, while bi-antennary glycans are more predictive for the occurrence of HCC relapse - independently of disease stage. Changes in serum glycans might thus provide an excellent framework for developing non-invasive prognostic biomarkers for the prediction of the two-year survival rate and relapse in HCC patients.

Figure:



A)



B)

Figure 1. Kaplan-Meier survival curves for the cumulative two-year survival rate according to the glycan composite score 1 (A) and the cumulative HCC-free survival (no HCC relapse) according to the glycan composite score 2 (B).

P04-19
Efficacy of Lenvatinib in intermediate stage and advanced hepatocelluara carcinoma: results of monotherapy and combination with locoregional treatment

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Background and Aims: Lenvatinib is the one of the two mainstay first-line therapy for unresectable Hepatocellular carcinoma (HCC). Combined treatment with locoregional treatment and Lenvatinib has been proposed as an alternative strategy in more advanced disease. Real-world study comparison of efficacy and safety between these two regimens is limited, previous studies suggested a higher response rate from locoregional plus systemic treatment than chemotherapy alone.
In this study, we aimed to evaluate the efficacy of lenvatinib in intermediate and advanced stage HCC.

Method: 46 patients with Child-Pugh score 5-7 and ECOG PS 0-1 who received Lenvatinib for Intermediate or Advanced stage HCC from April 2020 to October 2022 at our hospital were enrolled. First, landmark analyses were performed to evaluate the association between radiological response and prognosis. Univariate and multivariate analyses were then performed to search for factors contributing to OS and Response Rate.

Results: Median age was 72,5 years, Child-Pugh score was 5 in 35 patients, 6 in 5 patients, 7 in 6 patients. 35 patients were started in the first line Lenvatinib and 11 patients were treated with TACE/TARE during the period of lenvatinib. The median OS in 46 patients were 47 weeks, and the ORR was 62 %, in the best response by mRECIST. In a landmark analysis comparing the responder and non-responder groups, the complete responder group (12%) was treated with combined treatment. Multivariate analysis showed that relative tumor volume, AFP value before induction, first response, and combination of Lenvatinib with locoregional treatment were independent predictors of OS.

Conclusion: The efficacy of lenvatinib in the treatment of intermediate/advance stage HCC was favorable. The combination of lenvatinib and locoregional treatment may improve further prognosis, even in those patients who are refractory or unsuitable for TACE.

P04-20
Is ALBI score alone a good predictor for outcome in patients treated with radioembolization for hepatocellular carcinoma?

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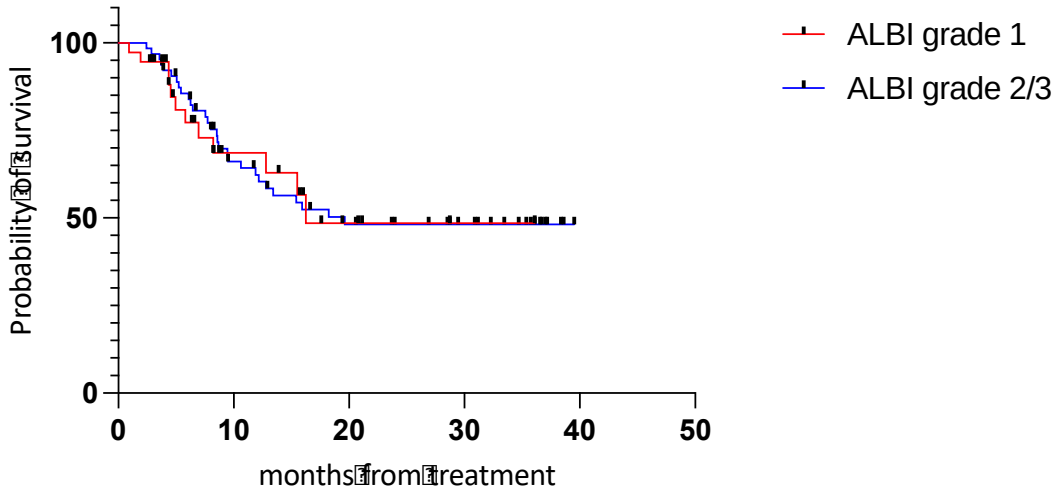
Background and Aims: Hepatocellular carcinoma (HCC) is the most common primary liver tumor and the third most common cause of cancer mortality worldwide. Transarterial radioembolization (TARE) with yttrium 90 has increasingly been performed to treat hepatocellular carcinoma (HCC). It can be recommended to patients with a bulky tumor and/or portal vein invasion. The integration of albumin-bilirubin (ALBI) grade is emerging to assess liver function on account of its objectivity and reproducibility. Our aim was to investigate the value of ALBI grade in predicting the outcome in patients treated with TARE.

Method: We retrospectively enrolled patients (n=104) with advanced and unresectable HCC treated with TARE at our institution from October 2019 until November 2022. Data collected included albumin, creatinine, bilirubin, INR, alpha-fetoprotein (AFP), MELD-Na (Model for End-Stage Liver Disease) from blood tests done one or two days before the procedure. All patients underwent a preliminary dosimetric study before Yttrium-90 resin microsphere TARE. Overall survival after TARE was assessed with Kaplan-Meier method. Survival analyses were stratified according to gender, ALBI grade, AFP, etiology and MELD-Na.

Results: 101 patients were enrolled in the study. 3 patients were excluded from the analysis for incomplete biochemistry data. Mean age at TARE was 66.71 ± 10.09 years ; 85 males (84%) and 16 females (16%). Mean survival was 14.54 months. Median AFP was 16.9 ng/ml. In terms of survival there was not a statistically significant difference between patients with an ALBI grade 1 compared with those with an ALBI grade 2 or 3. ALBI grade 1 patients (n=37) had statistically significant higher AFP values compared with ALBI grade 2 or 3 patients (n= 64) when using a cut-off of 100 ng/ml (Fisher's exact test, p 0.01). Survival was not significantly different stratified for gender, etiology, MELD Na.

Conclusion: In our cohort ALBI score alone does not appear to predict survival in patients treated with TARE for HCC. This might be accounted to the fact that ALBI grade 1 patients had a significantly higher AFP values compared with ALBI grade 2 or 3 patients. Our study supports the use of AFP, in addition to ALBI score, to discriminate patients that might be worse candidates for TARE, helping to guide treatment decisions.

Figure:


100
50
0
0
10
20
30
40
50
months from treatment
ALBI grade 1
ALBI grade 2/3

P05-01-YI
Macrotrabecular-Massive pattern is associated with aggressive factors, but it is not an independent predictor of tumor recurrence and overall survival in patients with hepatocellular carcinoma treated with liver resection

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Background and Aims: The assessment of recurrence risk after liver resection (LR) is critical, particularly with the advent of effective adjuvant therapy. The presence of macrotrabecular-massive histological pattern (MTM: trabeculae >6 cells thick and whose pattern represents >50% of the tumor) has been recently associated with an increased risk of tumor recurrence and decreased overall survival (OS) after LR in patients with hepatocellular carcinoma (HCC). The aim of the study was to describe the subgroup of patients with HCC-MTM, as well as to evaluate its association with aggressive recurrence and OS after LR.

Method: Retrospective study in which all BCLC-0/single-A patients treated with LR between February 2000 and November 2020 were included. The main clinical variables were recorded at the time of admission, surgery and during follow-up. Histological features were evaluated by two independent pathologists. Aggressive recurrence was defined as those that exceeded the Milan criteria at the 1st recurrence.

Results: A total of 218 patients were included (30% BCLC 0 and 70% BCLC A), the median (interquartile range) age and tumor size were 63 years (54-69) and 28 mm (19-42), respectively. The prevalence of MTM and microvascular invasion and/or satellitosis (mVI/S) was 7.8% and 39%, with a kappa index between the two pathologists of 0.81 and 0.77. The presence of the MTM histological pattern was significantly associated with a higher prevalence of mVI/S (82.4% vs. 35.8%, p<0.001), Edmonson Steiner grade III-IV (82.4% vs 36.8%, p<0.001), and higher AFP values [185 (7-664) vs 6 (3-20) ng/mL, p<0.001]. The median follow-up to the 1st recurrence was 34 (14-59) months and 127 (58%) patients experienced HCC recurrence. The recurrence free survival was 51 (41-61) months. The prevalence of aggressive recurrence was 35% (44/127, advanced stage in 20). The median follow-up was 49 (23-85) months, with a 5-year OS of 81%. The presence of MTM was not significantly associated with recurrence [HR: 1.57 (0.84 - 2.92), p:0.154] or with aggressive recurrence [HR: 1.57 (0.53 - 4.43), p:0.154], and the presence of mVI/S was the only independent predictor of aggressive recurrence [HR: 3.31 (1.74 – 6.29), p<0.001]. Regarding OS, in the univariate analysis the presence of MTM was associated with a higher risk of death [HR: 2.50 (1.19-5.29), p:0.016], but after adjusting for AFP and mVI/S, it was not a predictor of OS [HR: 1.32 (0.49 - 3.52), p:0.573]. The presence of mVI/S was the only independent predictor of mortality [HR: 2.23 (1.27- 3.91), p:0.005].

Conclusion: The MTM pattern is significantly associated with clinical and histological features of poor prognosis (worse degree of differentiation, mVI/S, and elevated AFP values), but it does not represent an independent predictor for recurrence/OS. Finally, the presence of mVI/S was the only independent risk factor of aggressive recurrence or lower OS rate.

P05-02-YI
Feasibility of systemic anti-cancer therapy as an alternative to best supportive care in patients with advanced HCC and Child-Pugh B liver dysfunction

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Background and Aims: No randomized controlled trial evidence exists to support use of systemic therapy in patients with hepatocellular carcinoma (HCC) in the setting of liver dysfunction. Although selected patients with Child-Pugh (CP) B cirrhosis can likely be safely treated with immunotherapy, whether systemic anti-cancer therapy (SACT) improves survival in this population is unclear. In this retrospective study, we described outcomes of CP-B patients treated with either sorafenib (Sor), nivolumab (Nivo) or atezolizumab plus bevacizumab (A+B) and compared them to best supportive care (BSC) in a propensity score weighted (PSW) analysis.

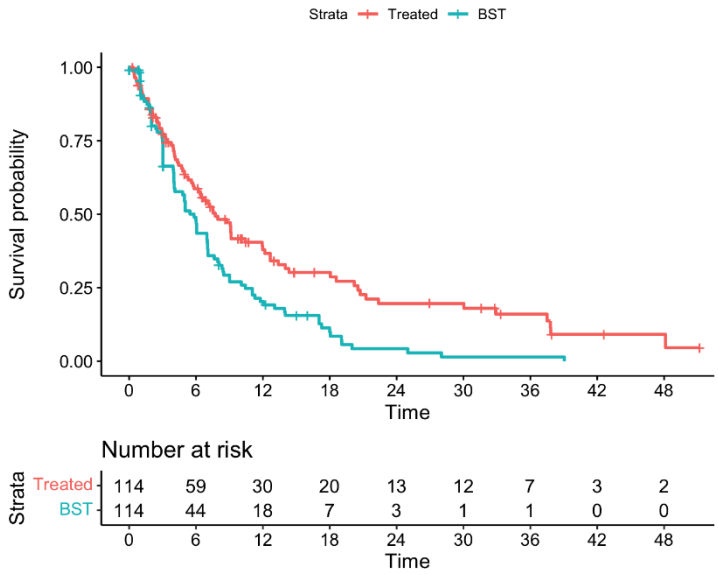
Method: From two international consortia, we selected CP-B patients receiving A+B (n=72), Nivo (n=46) or Sor (n=114) as first-line systemic therapy for advanced HCC between 2010 and 2022 and compared outcomes to a cohort of 159 patients receiving BSC in the same timeframe. SACT exposure was evaluated in relationship to OS using propensity score weighted (PSW) multivariate regression models.

Results: In the unmatched population (n=392), the 233 patients receiving active treatment for HCC had a longer mOS compared to the 159 receiving BSC (6.5 vs. 5.5 months, p=0.011).

Following PS matching for Barcelona Clinic Liver Cancer (BCLC) stage (A/B vs C), alpha-fetoprotein (AFP) (>400 vs <=400 ng/mL), CP score (7/8 vs 9) and presence of main portal vein tumor thrombosis (PVTT), 114 couples were left, and receipt of SACT remained associated with improved mOS (7.7 vs 5.8 months, p<0.001).
PSW univariate regression analyses demonstrated a significant reduction in the risk of death in patients exposed to A+B (HR: 0.6, 95%CI: 0.4-0.8); Nivo (HR: 0.5, 95%CI:0.4-0.8) and Sor (HR: 0.6, 95%CI: 0.5-0.9, p=0.001). Better OS was observed in patients exposed to A+B (HR: 0.6, 95%CI: 0.4-0.8), Nivo (HR: 0.4, 95%CI:0.3-0.7) and Sor (HR: 0.7, 95%CI: 0.6-0.9, p<0.001) following adjustment for unbalanced prognostic traits in PSW-multivariate models.
Significantly inferior survival was observed in patients with AFP >400 ng/mL (HR: 1.9, 95%CI: 1.5-2.5); CP score of 9 (HR: 1.7, 95%CI: 1.2-2.4) and in the presence of PVTT (HR: 1.8, 95%CI: 1.4-2.4).
In the SACT-exposed group, incidence of treatment-related adverse events (trAEs) of all grades was 59%. trAEs rates were higher in A+B (69%) and Sor (67.5%) than Nivo (43.2%, p<0.001).

Conclusion: Receipt of SACT was associated with a small but significant OS improvement in CP-B patients treated with SACT compared to BSC. PS-weighted OS estimates indicate extended survival times in patients with CP 7-8, AFP<400ng/mL and no PVTT, identifying a patient sub-population where SACT may be delivered with higher clinical benefit. This retrospective study provides a benchmark for the survival extension potentially associated with SACT delivered in CP-B patients.

Figure: KM curves for OS in the PS matched population.



P05-04
Cryptogenic non-cirrhotic HCC: clinical, prognostic and immunologic aspects of an emerging HCC etiology

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Background and Aims: The incidence of hepatocellular carcinoma (HCC) in non-cirrhotic livers is increasing. In order to better understand this trend, we conducted a comprehensive study to investigate the characteristics of HCC in non-cirrhotic livers in detail.

Method: Data was analyzed of 2304 HCC cases diagnosed at a large referral center in the Netherlands between 2009 and 2020, and 1654 cases with a complete medical record were included for analysis. Patient characteristics, liver disease etiologies, post-diagnosis survival rates, genetic risk factors, and immune profiles were analyzed.

Results: Of the 1654 included HCC cases, 371 (22%) were non-cirrhotic. The incidence of non-cirrhotic HCC rose by 61% between 2009 and 2020, with 39% of cases diagnosed in the absence of underlying liver disease classified as cryptogenic non-cirrhotic HCC. Cryptogenic non-cirrhotic HCC cases were similar to non-cirrhotic NAFLD HCC cases in terms of patient characteristics, but had more advanced tumors, a higher prevalence of symptoms (such as significant weight loss) at the time of diagnosis, and shorter survival times. Overall survival of non-cirrhotic cryptogenic HCC was dismal compared to viral and non-viral causes of HCC (figure 1). In a multivariable analysis, cryptogenic etiology was found to be an independent negative prognostic factor (p=0.037), along with intermediate and advanced tumor stage and older age. More advanced stages of cryptogenic HCC were associated with higher levels of circulating interleukin-6. Analysis of a balanced sub-cohort of non-cirrhotic cryptogenic and NAFLD HCC cases revealed comparable immune profiles and HCC risk gene phenotypes.

Conclusion: These findings suggest that cryptogenic non-cirrhotic HCC may represent a unique HCC etiology, with more aggressive traits such as advanced tumors and a pro-inflammatory immune protein signature in the blood. These observations made us postulate that cryptogenic non-cirrhotic HCC may be a group of patients with "burned-out" NAFLD. Further research is needed to identify risk factors and guide better clinical management.

P05-09
Evaluation of ESPL1 level in peripheral blood of patients with HBV infection for early warning of HCC occurrence and recurrence

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Background and Aims: Previous studies have found that the expression level of the extra spindle pole bodies-like 1 (ESPL1) gene is increased in a variety of malignant tumour tissues. Hepatitis B virus (HBV) infection is the main cause of hepatocellular carcinoma (HCC), and the development of HCC usually follows the route of chronic hepatitis B (CHB), liver cirrhosis (LC) and HCC. In this study, ESPL1 levels in peripheral blood of patients with CHB, LC and HCC were compared to observe the relationship between ESPL1 and the progression of liver disease. In addition, the peripheral blood ESPL1 level in HCC patients was dynamically observed to explore the value of ESPL1 for the diagnosis and early warning of HCC.

Method: A total of 636 patients infected with HBV were enrolled in this study, all of whom were from our long-term follow-up cohort of HBV infection, including 333 patients with CHB, 195 patients with LC and 108 patients with HCC. 42 controls were from healthy people. ESPL1 level in peripheral blood of each group was detected by enzyme-linked immunosorbent assay (ELISA). In addition, ESPL1 level in peripheral blood of 33 HCC patients with a median follow-up time of 11.8 years before and after hepatectomy was dynamically detected to analyze the relationship between the occurrence and recurrence of HCC with ESPL1 level.

Results: The average levels of ESPL1 in peripheral blood of healthy control group, CHB group, LC group and HCC group were (131.622±41.523) ng/L, (216.712±128.761) ng/L, (296.024±153.158) ng/L and (567.084±414.296) ng/L respectively ($P < 0.05$ for any two groups), showing a trend of increasing synchronously with the progression of liver disease. Taking the average value of ESPL1 level in peripheral blood of 33 HCC patients at the corresponding time points, we found that the average value of ESPL1 was at a low level before HCC occurrence, reached the highest level when HCC occurred, decreased after hepatectomy (Figure 1), but increased again when HCC recurred (Figure 2).

Conclusion: The level of ESPL1 in peripheral blood of HBV-infected patients increase simultaneously with the progression and severity of liver disease, which shows a synchronous fluctuation trend of increasing, decreasing and increasing again with the occurrence, hepatectomy and recurrence of HCC. The changes of ESPL1 level in peripheral blood of HBV-infected patients have diagnostic and early warning value for HCC occurrence and recurrence.

Figure:

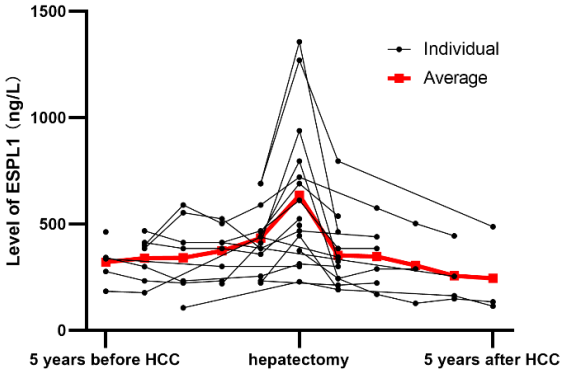

Figure 1. Changes of ESPL1 level in peripheral blood of HCC patients without recurrence after hepatectomy.

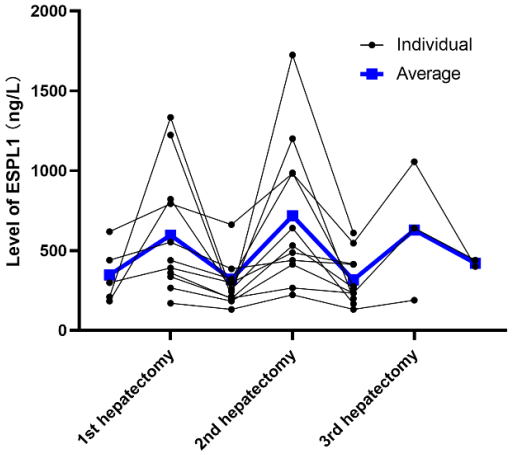

Figure 2. Changes of ESPL1 levels in peripheral blood of HCC patients with recurrence after hepatectomy.

P05-11
The epidemiology of hepatocellular carcinoma has changed in our region ... But, what has really changed ?

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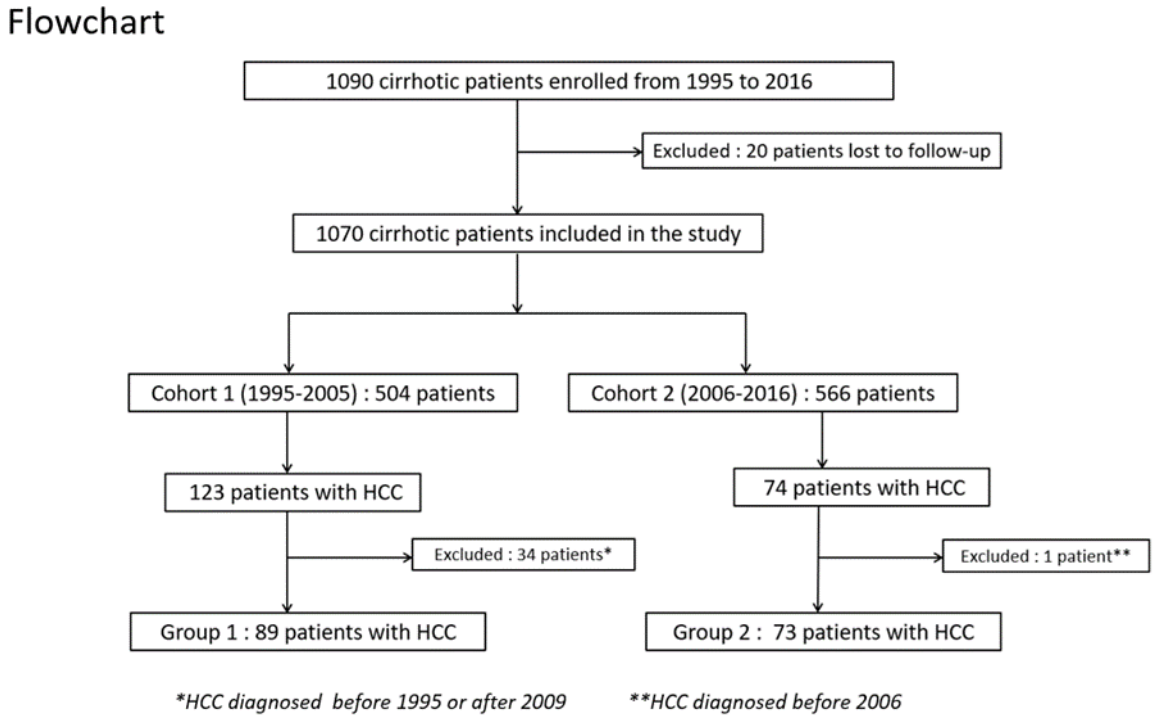
Background and Aims: The epidemiology of cirrhosis has changed over the last two decades in our institution. The aim of this study was to assess whether the epidemiology and clinical presentation of hepatocellular carcinoma (HCC) occurring in cirrhosis has also changed during the last 25 years.

Method: Patients were recruited from the Cirrhosis Registry. This database included patients with cirrhosis who had attended the outpatient' liver clinic at the Centre Hospitalier Jolimont, in La louvière, Belgium since January 1995. We extracted data on two cohorts of patients with cirrhosis collected over an identical time period (11 years) and followed up for the same duration, but 11 years apart.

Results: Cohort 1 included 504 patients enrolled from 1995 to 2005, among them, 89 patients developed HCC during the defined follow-up period (group 1). Cohort 2 included 566 patients enrolled from 2006 to 2016 among whom 73 developed HCC during the defined period of follow-up (group 2). When patients with HCC in both groups were compared, no differences were found in the age at HCC diagnosis, the test that alerted on the presence of HCC, the extension and stage of the lesion at diagnosis. In the group 1, hepatitis C virus-related HCC occurred in more than half of the cases (53%) compared with 18% in the group 2 ($P<0.001$). Alcohol-related HCC occurred in 27% in the group 1, compared with 60% in the group 2 ($P<0.001$). In contrast, the prevalence of metabolic-associated fatty liver disease-related HCC accounted for approximatively 10% in all groups.

Conclusion: The general epidemiology of HCC has not changed; however the etiology of underlying cirrhosis has changed.

Figure:


Flowchart
1090 cirrhotic patients enrolled from 1995 to 2016
Excluded : 20 patients lost to follow-up
1070 cirrhotic patients included in the study
Cohort 1 (1995-2005) : 504 patients
Cohort 2 (2006-2016) : 566 patients
123 patients with HCC
74 patients with HCC
Excluded : 34 patients*
Excluded : 1 patient**
Group 1 : 89 patients with HCC
Group 2 : 73 patients with HCC
*HCC diagnosed before 1995 or after 2009
**HCC diagnosed before 2006

P05-12
Clinical experience of diagnostic examination of hepatocellular carcinoma

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Background and Objectives: hepatocellular carcinoma (HCC) is the most sinister complication of chronic hepatitis, liver cirrhosis (LC) of various causes, and non-alcohol fatty liver disease (NAFLD). Ukraine highest HCC morbidity (up to 5,2%). Usually, HCC is diagnosed incidentally on US, MDCT and MRI. In 2017 American college of radiology (ACR) introduced the program for HCC screening, diagnostic, and active surveillance. Objectives: role and efficacy assessment of HCC US LI-RADS (US LR) diagnostic screening ultrasound algorithm.

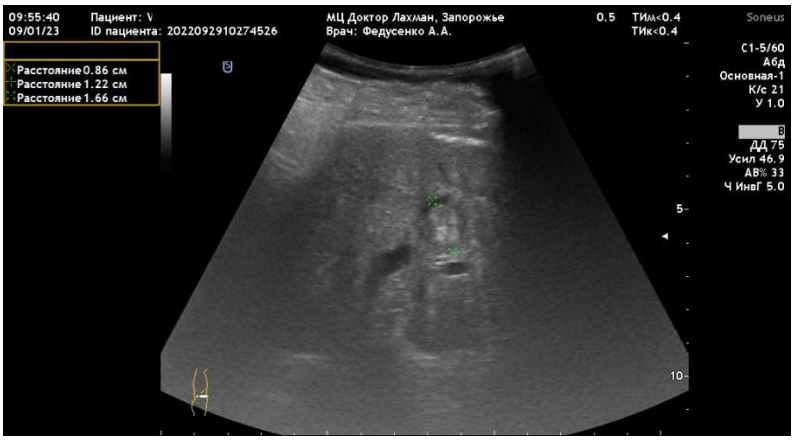
Methods: to assess the efficacy of US screening and active surveillance diagnostic algorithm, abdominal mp-US data of 4299 patients at risk for developing HCC have been analyzed from 2017 to 2022. Among them: 828 patients with LC of various etiology, 461 patients with hepatitis B (HBV) without LC, and 3010 patients with hepatitis C (HCV). Based on US-elastometry (2D-SWE), this patient cohort had 1100 cases with high-grade fibrosis (F3 and F4, according to METAVIR). Alfa-fetoprotein level (AFP) was determined in all patients with focal liver observations.

Results: 285 cases with focal liver observations were found in the cohort with high-grade fibrosis and LC. US LR was exploited to further select patients for contrast-enhanced cross-sectional imaging. US LR is a standardized system for US-images processing, interpretation, reporting, and collecting data for screening or active surveillance of patients at risk for developing HCC.

226 patients, based on mp-US data, were qualified as US LR3. These patients underwent contrast-enhanced MDCT and/or MRI, thereby 27 HCCs were diagnosed (11,9%). Lesions were further verified morphologically with core biopsy under US guidance. In addition, due to high-specific enhancement patterns, MDCT and MRI allowed to verify 155 cases of hepatic hemangioma (68,5%; either flesh-filling or cavernous), focal nodular hyperplasia (7 patients; 3,1%), hepatic adenoma (5 patients; 2,2%).
All 27 patients with HCC had raised AFP levels (more than 15 times above the normal level).

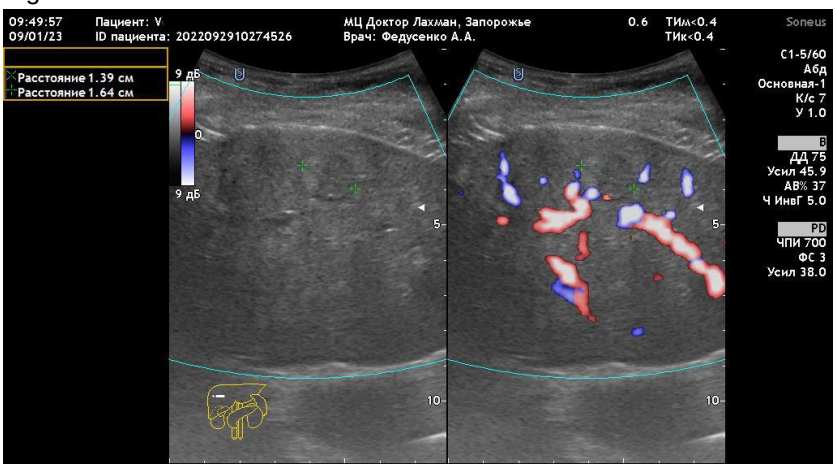
Conclusion: diagnostic algorithm US LI-RADS for HCC screening and active surveillance allows standardization of the US application in patients at high risk for HCC. US LR complements current standardized systems of HCC contrast-enhanced cross-sectional imaging (LI-RADS CT/MRI and CEUS LI-RADS). Contrast-enhanced imaging should always be used in all US LR3 patients. Additional information about AFP levels allows laboratory confirmation of suspected HCC, improving accuracy. Introducing the US LR system in the daily practice of ultrasound specialists is appropriate.

Figure 1.



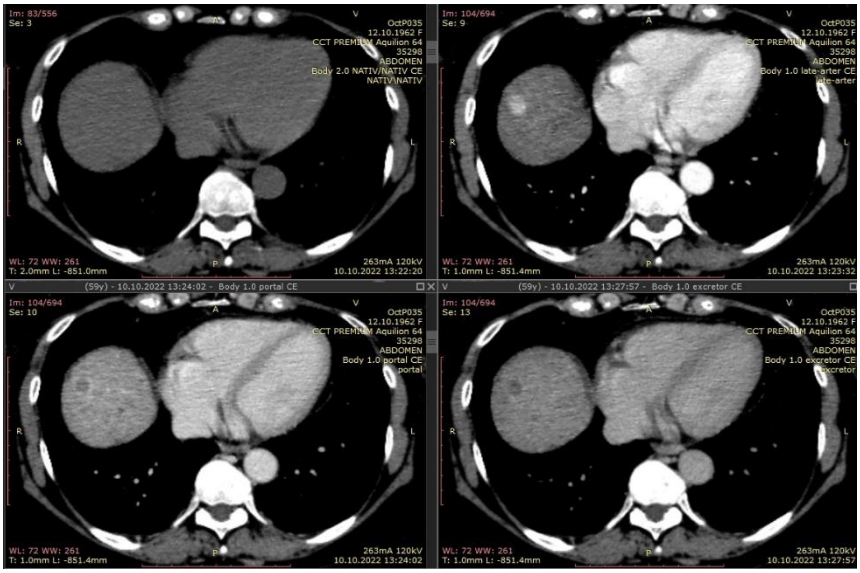
2D scan. US LI-RADS-3, heteroechogenic focus in S8 liver Ø 16 mm. Histological: well-differentiated hepatocellular carcinoma, G1.

Figure 2.



Power Doppler mode. Same patient.

Figure 3.



MDCT. Same patient. Multiphase scanning. The classic perfusion pattern for HHC.

P05-14
The role of radiomics in diagnosis and therapeutic path of hepatocellular carcinoma treated with transarterial chemoembolization

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Background and Aims: Radiomics is an innovative technology used to quantify the heterogeneity of tumors through the analysis of features, related to spatial distribution of grey level in medical imaging. Recently, the application of radiomics in hepatocellular carcinoma has shown encouraging results. Studies have demonstrated that radiomics can predict both the biological and the molecular profile of the tumor, together with the response to therapy and the outcome.
The aim of our study is to evaluate:

- The variation of clinical and laboratory parameters after trans arterial chemo embolization (TACE);
- The variation of radiomic features after TACE;
- The correlation between radiomic features before and after TACE and radiologic response, evaluated through mRECIST criteria;
- The correlation between radiomic features before and after TACE and survival.

Method: We considered the arterial phase of CT images of a population of 22 patients, diagnosed with hepatocellular carcinoma, belonging to Child-Pugh class A or B and treated with TACE. We were able to extract and analyze 155 radiomic features, using the LIFEx program.

Results: After TACE, patients showed a significant worsening of Child-Pugh score (from 6.14 to 6.8, p-value 0.023) and a reduction of the mean size of the tumor (from 28.09 mm to 21.09 mm, p-value 0.005). Radiomic texture and intensity features showed a significant variation after TACE (p-value < 0.05). We demonstrated a strong correlation between some morphology, intensity and texture parameters before (table 1) and after TACE and the response to the treatment, evaluated through mRECIST criteria (p-value < 0.05). Just one morphology feature extracted from CT images before TACE was found to be a statistically significant predictor of survival of patients.

Conclusion: This study suggests that radiomics can be a reliable technique in predicting the response to therapy and the survival in patients with hepatocellular carcinoma treated with TACE. Future studies need to investigate the existence of other radiomic features to better characterize the response to treatment and to predict the survival after TACE.

Table:

1.Radiomic features before TACE which correlate with response to treatment evaluate with mRECIST criteria.

RADIOMIC FEATURE (media± SD)	M RECIST criteria				RELABILITY (p-value)
	CR	PR	SD	PD	
MORPHOLOGICAL Volume IBSI RNU0	9231,024 ± 6695,5	26359,29 ± 34535,62	3989,565 ± 5142,63	294334,5 ± 0	0,00004
INTENSITY HISTOGRAM Intensity Histogram Minimum Grey Level HU IBSI 1P R8	98,88889 ± 4,22	97,6 ± 4,29	100 ± 1,41	76	0,00089
NGTDM Coarsenes s IBSI QCDE	0,002295418 ± 0,002	0,001530145 ± 0,001	0,01233688 ± 0,01	4,475025E- 05	0,01915

P05-15
Hepatocellular carcinoma incidence and risk stratification in patients with non-alcoholic fatty liver disease on long-term follow-up

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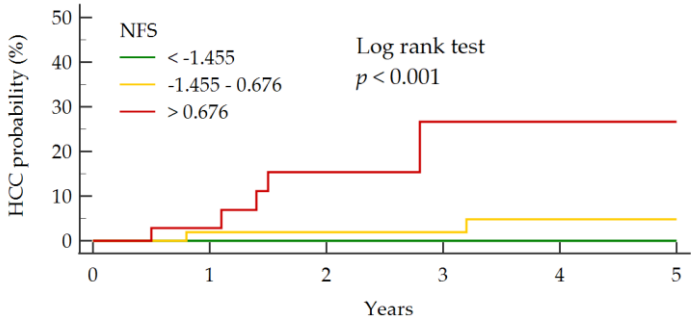
Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is projected to become the leading cause of hepatocellular carcinoma (HCC) worldwide. However, HCC surveillance in patients with NAFLD is challenging due to the high disease prevalence, the tumour occurrence even in the absence of liver cirrhosis, and the limitations of ultrasound (US) in such patients. The aim of the study was to investigate the incidence of HCC in NAFLD patients, and to assess the performance of non-invasive tests (NITs) for the stratification of the risk of HCC development in patients with NAFLD on long-term follow-up (FU).

Method: We retrospectively enrolled 185 patients with biopsy-proven NAFLD or clinical diagnosis of NAFLD-related liver cirrhosis (F2=51, F3=55, F4=79). All patients had at least 6 months FU with regular US surveillance (annually in F2 and biannually in F3/F4). The following NITs were calculated at liver biopsy or at clinical diagnosis of liver cirrhosis: AST to ALT ratio (AAR), AST to platelet ratio index (APRI), Fibrosis-4 score (FIB-4), BARD score, NAFLD fibrosis score (NFS), and age–male–ALBI–platelets (aMAP) score.

Results: No HCC occurred in patients with F2 (37 [72.5%] males; age 47 [39–58] years; BMI 29.3 [26.5–31.6] kg/m^2; T2DM 14 [27.5%]) during a median FU of 4.9 (IQR 1.3–7.3) years. Conversely, 11 *de novo* HCC occurred in the 134 patients with F≥3 (65 [48.5%] males; age 58 [50–66] years; BMI 31.0 [28.0–33.2] kg/m^2; T2DM 77 [57.5%]) during a median FU of 3.2 (IQR 1.4–6.0) years. Notably, only 1 HCC occurred in F3 patients (incidence rate: 0.4 per 100 person/years), while 10 HCC occurred in patients with liver cirrhosis (incidence rate: 3.4 per 100 person/years). BCLC stage was 0 in 4 (36.4%) patients, A in 4 (36.4%), B in 2 (18.2%), and C in 1 (9.1%). In the subgroup of 134 patients with F≥3, all the NITs were significantly associated to an increased risk of HCC development (AAR: HR=5.08, p=0.012; APRI: HR=1.74, p=0.011; FIB-4: HR=1.14, p<0.001; BARD: HR=1.72, p=0.049; NFS: 1.98, p<0.001; aMAP: HR=1.14, p=0.002). The best accuracy for HCC prediction was observed for NFS and aMAP (c-index=0.80, both), followed by FIB-4 (c-index=0.77), APRI (c-index=0.72), AAR (c-index=0.66), and BARD (c-index=0.63). Among the different NITs investigated, NFS allowed to effectively stratify patients into 3 different risk categories with distinctly different HCC incidence: NFS<-1.455 = 0/39 (0%), NFS between -1.455–0.676 = 3/60 (5.0%), and NFS>0.676 = 8/35 (22.9%) (Log-rank test: p<0.001) (Figure 1).

Conclusion: In NAFLD patients on long-term FU, HCC mostly develops in the presence of advance liver disease.Several NITs (i.e. NFS, aMAP, and FIB-4) showed an appropriate performance for the prediction of HCC development. In particular, NFS may be useful to tailor personalized surveillance strategies in patients with NAFLD and advanced liver disease.
Funded by EU/EFPIA-IMI2 g.a. no.777377, LITMUS

Figure:



Number at risk

Group: < -1.455					
39	36	30	23	21	16
Group: -1.455 - 0.676					
60	48	40	34	24	19
Group: > 0.676					
35	24	15	11	7	5

P05-16
Prognostic role of thyroid volume reduction in patients treated with lenvatinib for advanced hepatocellular carcinoma

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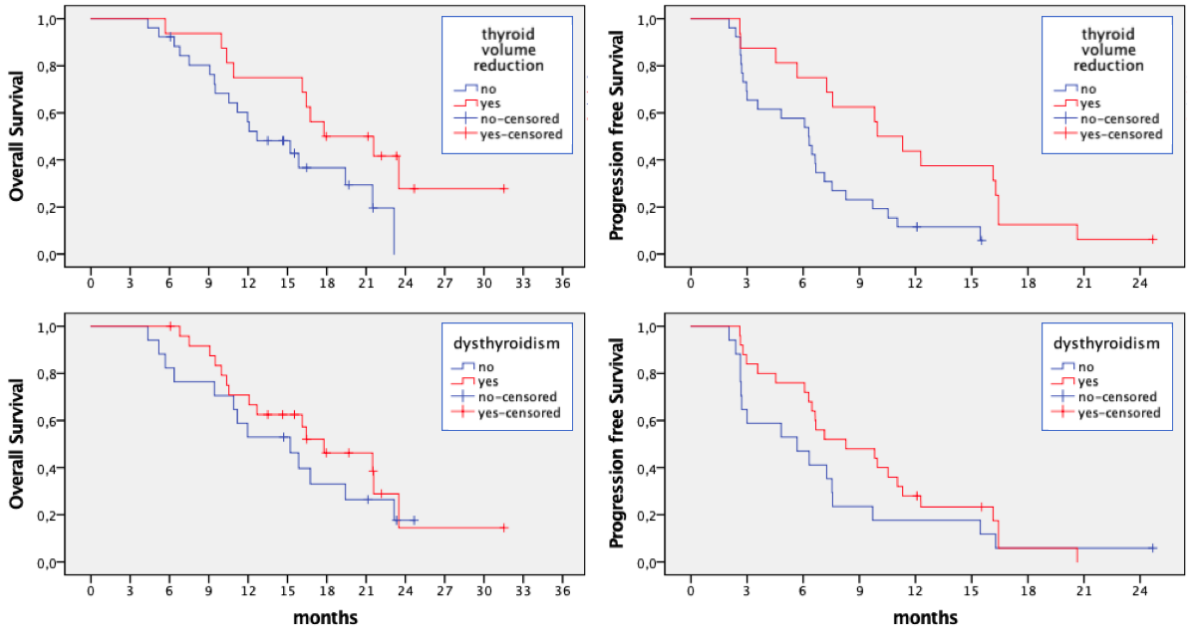
Background and Aims: Lenvatinib is a multiple tyrosine kinase inhibitor approved as first-line therapy for advanced HCC (HCC). Among drug-related adverse events, hypothyroidism is very common. Two recent studies investigated the prognostic role of occurrence of dysthyroidism during treatment with lenvatinib in term of overall survival (OS) and progression free survival (PFS). It is not known if lenvatinib may cause thyroid gland dimension variations and if this could have a prognostic effect.

Method: We retrospectively enrolled all patients treated with lenvatinib for advanced HCC from March 2020 to March 2022 in four different Italian prescribing centers. Thyroid gland volume has been measured at baseline and at further radiological re-evaluations with CT scan according to clinical practice. We considered a reduction in thyroid volume >10% from baseline as significant. Closure of follow up was 31st January 2023.

Results: The study population consisted of 42 patients. 25 patients (59.5%) presented dysthyroidism and 16 patients (38.0%) presented a reduction in thyroid volume during treatment. In survival analysis, thyroid gland volume reduction of >10% from baseline was significantly related to longer OS (17.7 vs 12.6 mo; p=0.030) and PFS (9.9 vs 6.2 mo; p=0.018). Conversely, occurrence of dysthyroidism during treatment did not statistically correlate with neither OS (17.7 vs 15.2 mo; 0.375) nor PFS (8.2 vs 5.6 mo; p=0.237). The disease control rate at 3 months (DCR-3) of patients with thyroid volume reduction was significantly higher (93.7% vs 65,3%; p=0.036); among this subgroup of patients, a sustained disease control rate at 6 months (DCR-6) remained higher in patients with thyroid volume reduction (80.0% vs 41.1%; p=0.026).

Conclusion: Thyroid gland volume reduction during treatment with lenvatinib seems to be a predictor of response and survival for patients with advanced HCC. Dysthyroidism does not seem to be related to prognosis in these patients.

Figure:


Overall Survival
thyroid volume reduction
no
yes
no-censored
yes-censored
Progression free Survival
thyroid volume reduction
no
yes
no-censored
yes-censored
Overall Survival
dysthyroidism
no
yes
no-censored
yes-censored
Progression free Survival
dysthyroidism
no
yes
no-censored
yes-censored
months
months

P05-17
Liver function is a predictor of survival in patients with hepatocellular carcinoma in best supportive care

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Background and Aims: The prognosis of patients with hepatocellular carcinoma (HCC) is very variable, and the relative contribution of tumor burden and liver dysfunction to survival is uncertain. Median overall survival (OS) of patients managed with best supportive care is around 3-6 months, although longer values may be observed in clinical practice. Aim of this study was to identify the factors linked to tumor or liver dysfunction associated with survival in patients with HCC treated with BSC.

Method: We retrospectively evaluated the clinical characteristics of 1414 patients who had an indication for BSC recorded in the Ita.Li.Ca. database between 2000 and 2020. We analyzed both patient and tumor characteristics to identify predictors of OS.

Results: Median age was 69y and 76% of patients were male. 67.4% of patients had a performance status 0-1 and 41.4% were in Child-Pugh B class. Median MELD was 13. 60% of patients had a multifocal HCC with a median number of 3 lesions and a median size of 33mm. 533 patients had vascular invasion. Median alpha-fetoprotein was 49.25 ng/ml. 111 patients were classified as BCLC-A, 148 as BCLC-B, 791 as BCLC-C and 325 as BCLC-D (12 unknown). Among comorbidities, obesity (p<0.001), hypercholesterolemia (p=0.036) and hypertriglyceridemia (p=0.034) were associated with lower OS. Absence of symptoms (6 vs 10 months, p<0.001), lack of vascular invasion (9.1 vs 5.03, p<0.001), and absence of metastasis (8.167 vs 4.733 p<0.001) were associated with a better OS. Median OS in BCLC-A (14.37 months) patients was longer than in stages B (9.20) or C (7.13). Survival progressively declined according to severity of liver function using three different scores (CPS, ALBI, pALBI, p<001).
Women tended to survive longer 23 vs. 19 months, p=0.053). Comparing patients surviving more or less than 12 months (398 vs. 1016), age, size of lesions, albumin, bilirubin, alpha-fetoprotein, and MELD were significantly different. At Cox univariate analysis presence of cirrhosis (HR: 1.201 CI 95%CI 0.998-1.445 p=0.052), number of lesion (HR: 1.02 CI 95%CI 1.01-1.04 p=0.013), median size (HR: 1.02 CI 95%CI 1.01-1.03 p<0.001), vascular invasion (HR: 1.80 CI 95%CI 1.59-2.02 p p<0.001), metastasis (HR: 1.48 CI 95%CI 1.28-1.72 p<0.001), ALBI grade (ALBI 2 HR: 1.25 CI 95%CI 1.04-1.50 p=0.015 ALBI 3 HR :1.75 CI 95% 1.43-2.14 p<0.001), pALBI grade (pALBI 2 HR: 0.99 CI 95%CI 0.8241.202p=0.960 pALBI 3 HR :1.49 CI 95% 1.25-1.78 p<0.001), MELD (HR: 1.04 CI 95%CI 1.04-1.05 p p<0.001) and CPS (HR: 1.13 CI 95%CI 1.10-1.165 p<0.001) were significantly associated with OS. Using different models to avoid colinearity ALBI, pALBI, and CPS maintained an independent prognostic role on OS (ALBI HR 1.58 CI 1.26-1.98 p<0.001, pALBI 1.22 CI 1.01-1.49 p=0.43, CPS 1.12 CI 1.85-1.16 p< 0.001).

Conclusion: In a large series of patients with HCC in BSC, parameters of liver function are strongly associated with survival.

P05-18
What changed in hepatocellular carcinoma presentation in the last eleven years? Comparison of two sequential time series from a tertiary hospital

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Background and Aims: Hepatocellular carcinoma (HCC) is the sixth most common cancer and the second leading cause of cancer mortality worldwide. Hepatitis B virus (HBV) and hepatitis C (HVC) remain, at present, the most important worldwide risk factors for HCC. Nevertheless, universal HBV vaccination of new-borns and effective treatments for chronic HCV infection, will likely decrease the rates of viral-associated HCC. Our main aim was to analyse the changes in demographics and risk factors for HCC in the last 11 years.

Method: Retrospective single-centre study, including all patients with HCC diagnosis between 2011 and 2021 who have been referred to the multidisciplinary hepatic tumours team, in a Portuguese tertiary hospital. We compared two subgroups, according to the diagnosis date: Group 1 from 01/2011 to 06/2016; Group 2 from 07/2016 to 12/2021. We analysed the patients' demographics, the presence of liver cirrhosis aetiology of liver disease, Child-Pugh score, ECOG score; alcohol abstinence, ALBI score, the presence of portal vein thrombosis and the imaging appearance of HCC. We performed a T-test to analyse the mean difference between the two different groups.

Results: From the total of 173 patients included, 141 were males, with a mean age of 66 years at diagnosis. Liver cirrhosis was present in 96% of patients with alcohol as main aetiology (50%), followed by HCV infection (25%), HBV (10%) and nonalcoholic fatty liver (8%). Comparing the two series (Table 1), during the same length of time, the more recent one had more patients referred, patients had a worst performance status and there were more patients non-Child A. In what regards aetiology there was a percentual increase in NASH and alcohol-related cases, and a decrease in HBV and HCV related cases, although it did not reach statistical significance.

Conclusion: There seems to a trend for a change of HCC etiology, as expected, with a relative increase in fatty liver disease, either alcohol or non-alcohol related. The observation that patients are referred even with decompensated liver disease, and without a good *performance status*, may indicate that consideration is now given to the management of HCC even in more advanced states, since there is a large array of options, that that may include active palliative care.

Table 1:

		Total (173)	Group 1 (64) 01/2011 to 06/2016	Group 2 (109) 07/2016 to 12/2021	p-value
Age at diagnosis (mean)		66	67	66	0,798
Gender	Males	141 (82%)	47 (73%)	94 (86%)	0,036
ECOG 0		130	58 (90%)	72 (66%)	**0,001**
Liver cirrhosis		167	62 (96%)	105 (98%)	0,848
Child–Pugh score A		135	57 (89%)	78 (72%)	**0,003**
Liver cirrhosis main ethology	alcohol	86	26 (41%)	60 (55%)	0,587
	HCV	43	21 (33%)	22 (25%)	
	HBV	17	8 (13%)	17 (10%)	
	NASH	13	3 (4%)	10 (8%)	

P05-19
Circulating tumour DNA (ctDNA) as a prognostic biomarker for transarterial chemoembolisation (TACE) for hepatocellular carcinoma (HCC)

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Background and Aims: Outcomes for patients following TACE are variable, ranging from 4 to 21 months. Few validated prognostic markers exist. We explored the feasibility of ctDNA as a potential prognostic biomarker to TACE.

Method: 30 patients were diagnosed with HCC, and the mean age of the patients was 69 years; the majority were male (70%). The plasma samples were collected at the baseline and a median of 6 weeks (1-12 weeks) following TACE. Ultra-deep sequencing (6,000X depth) was conducted on 18 HCC-related cancer genes, including the TERT promoter. Kaplan-Meier survival and Cox regression analyses were used to determine any association between mutated genes and clinical outcomes.

Results: The median overall survival (OS) and progression-free survival (PFS) were 34 (95% CI: 22.35–45.60) and 11.6 (95% CI: 5.83–21.2) months in the whole cohort, respectively. 308 mutations were identified across the 18 genes analysed. 40% (123/308) were unique. CTNNB1 and ARID1A were the most frequently mutated genes, each accounting for 25%, followed by SF3B1 (20%) and TERT (18%). Notably, mutations in the TP53 and CTNNB1 genes were found to be mutually exclusive. No mutation was associated with the response to TACE. Mutations in SF3B1 were found to be a significant independent risk factor for shorter PFS, when adjusted for the other predictors of PFS (HR: 4.12, 95% CI:1.53-11.1, p = 0.005). Mutations in the CTNNB1, TP53, and KEAP1 genes were associated with OS, with the latter remaining a significant independent negative prognostic factor on multivariate analysis (HR: 5.08, 95% CI:1.15-22.4, p = 0.032).

Conclusion: The findings suggest that ctDNA profiling of known genetic drivers of HCC may serve as a valuable prognostic biomarker prior to TACE and may assist with the stratification of patients suitable for first-line systemic therapy.

Figure: Univariate and multivariate Cox regression analysis associated with overall survival and progression-free survival in HCC treated by TACE.

Predictor	Overall survival						Progression-free survival					
	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
	HR	95%CI	p	HR	95%CI	p	HR	95%CI	p	HR	95%CI	p
CTNNB1 mutation	3.82	1.42-10.3	**0.008**	4.1	1-16.8	0.05	1.96	0.89-4.33	0.1	1.54	0.55-4.36	0.4
TP53 mutation	3.39	1.14-10.1	**0.028**	3.56	0.97-13.1	0.056	2.07	0.82-5.24	0.13	2.96	0.98-8.92	0.053
KEAP1 mutation	3.54	1.29-9.74	**0.014**	5.08	1.15-22.4	**0.032**	0.84	0.34-2.1	0.7	1.02	0.28-3.69	0.95
SF3B1 mutation	1.04	0.42-2.57	0.95	1.21	0.42-3.47	0.7	2.82	1.2-6.62	**0.018**	4.12	1.53-11.1	**0.005**
Number of mutations ⩾ 10	2.48	0.97-6.34	0.058				1.77	0.77-4.05	0.2			
Age ⩾ 65	1.11	0.43-2.89	0.8				1.4	0.63-3.11	0.4			
A vs. C BCLC	4.69	1.04-21.3	**0.04**	11.7	1.02-134	0.048	1.9	0.51-7.09	0.3	2.3	0.46-11.5	0.3
Tumour size ⩾ 5 cm	1.3	0.46-3.68	0.6				1.7	0.73-3.95	0.2			
Viral vs. Non-Viral	1.24	0.48-3.2	0.7	3.05	0.60-15.4	0.2	0.68	0.3-1.53	0.3	0.9	0.33-2.48	0.8

P05-20
Disparities in access to timely diagnosis and treatment for hepatocellular carcinoma - need for standardisation across cancer types

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Background and Aims: Hepatocellular carcinoma (HCC) is the fifth most common cancer and second leading cause of cancer-related death globally. In Ireland, mortality from liver disease has increased 400% over the past 40 years; a contributing factor is a 300% increase in primary liver cancer, of which HCC comprises the majority of cases. This is in contrast to other chronic diseases and cancers, for whom improved outcomes have been seen. Target quality metrics for cancers at Cancer Centres in Ireland include time to definitive diagnosis and time to treatment as less than 20 days. We wanted to outline the timelines for HCC diagnosis and treatment at our centre.

Method: The Hospital Clinical Governance Department approved this study (CA 2023/034). Using local and tertiary referral center databases as well as discharge coding, all patients diagnosed with HCC in our hospital from Jan 2021 to Dec 2022 were identified. Diagnosed was based on imaging criteria or histology where performed.

Results: 34 patients were diagnosed with HCC during this period. 85%(29) were male and 15%(5) female, median age was 70 years(range 52-87). Cirrhosis was present in 88%(30) of cases. BCLC staging at diagnosis was stage 0 in 3%(1), stage A in 32%(11), stage B 24%(8), stage C in 15%(5) and stage D in 26%(9). HCC was detected by surveillance in (16) 47% or symptomatic in (18) 53%. Of those diagnosed at surveillance 63% were stage A or 0 compared to 12% being stage A or 0 if symptomatic at diagnosis. Upon diagnosis only 21%(7) were eligible for curative treatment and 44% (15) were deemed for supportive care only. The median time from a suspicious scan (ultrasound or CT) to diagnostic radiology or histology was 49 days (range 1-267). The median time from diagnosis to treatment was 95 days (range 20-374).

Conclusion: At our institution, the majority of HCC cases are diagnosed outside of surveillance, and are associated with advanced stage at diagnosis, with worse outcomes. Severe deficiencies in access to timely diagnostics and treatment for HCC are evident, and must be addressed urgently to improve survival and outcomes for patients.

P06-01-YI
A Notch-ligand producing niche drives the transition of pre-malignant biliary disease into cholangiocarcinoma

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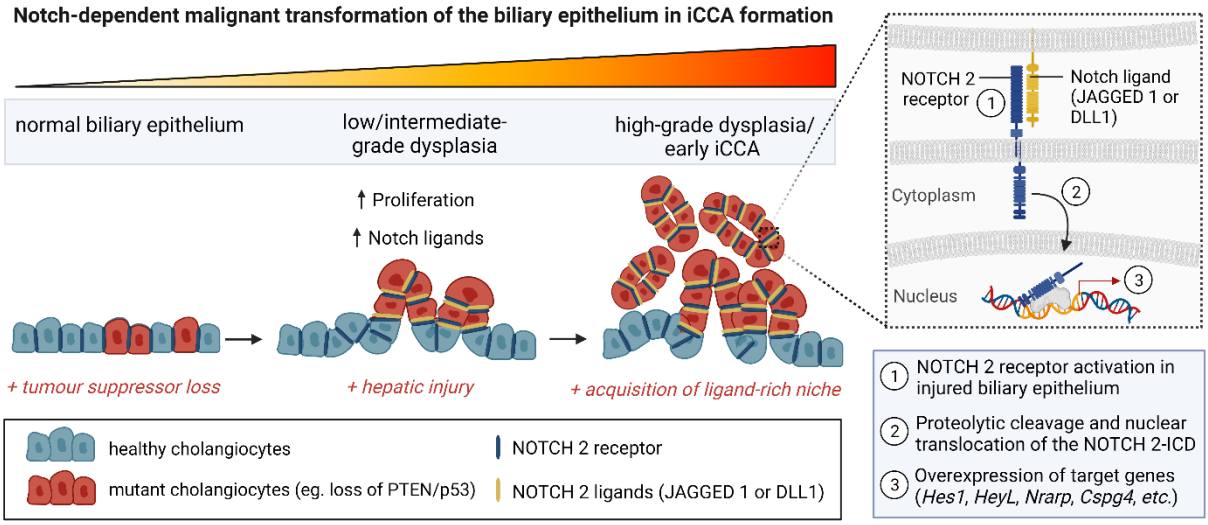
Background and Aims: Patients with primary sclerosing cholangitis or chronic infection with liver fluke (*O. Viverrini*) have a significantly elevated risk of developing intrahepatic cholangiocarcinoma (iCCA). We do not yet understand how such pre-malignant conditions transition into cancer, nor do we know what the molecular drivers of this process are. In this study, we aimed to **1)** characterise the molecular landscape at the interface between biliary disease and cancer, and **2)** test what cellular signals trigger the transition of the biliary epithelium into iCCA.

Method: We have developed a transgenic mouse model of iCCA called KPPTom (*Krt19-Cre-ER^{T}*;*Trp53$^{loxP/loxP}$*;*Pten$^{loxP/loxP}$*), which enables us to lineage-trace biliary cells as they undergo malignant transformation and iCCA formation. We used single cell RNA sequencing (scRNASeq), bulk RNA sequencing (RNA-seq), confocal microscopy, immunohistological analyses, and CRISPR/Cas9 gene editing to identify the molecular processes required for malignant transformation of the bile ducts and early iCCA growth.

Results: KPPTom mice fail to develop biliary tumours unless they also sustain cumulative hepatic injury, suggesting that an inflammatory microenvironment is necessary for iCCA formation. scRNASeq revealed that injured biliary cells transitioning into iCCA have a distinct transcriptional program compared to non-malignant cells, including the upregulation of Notch ligand genes *Jagged1* and *Dll1*. Immunohistological analyses of mouse and human iCCA livers confirmed that Notch ligands JAGGED1 and DLL1 are significantly enriched in tumour-initiating biliary cells, and that these ligands co-localise with high levels of activated (cleaved) NOTCH2 receptor at biliary cell interfaces. We ectopically activated Notch signaling in biliary cells lacking tumour suppressors *Pten* and *Trp53*, and found that Notch signalling was sufficient to initiate iCCA in the absence of hepatic injury. With hepatic injury, we observed a significant increase in tumour growth rate and the number of tumorigenic events. We isolated Notch-active cancerous biliary cells, and using RNA-seq, we identified a novel Notch-regulated transcriptional signature including *Hes1*, *HeyL*, *Nrarp* and *Cspg4*, amongst other genes. Using Cas9/CRISPR-mediated gene silencing, we interrogated whether these factors drive tumour initiation in a second Notch-driven mouse model of iCCA.

Conclusion: Our data demonstrates that during iCCA initiation, biliary cells form a cell-type autonomous Notch signaling niche that cooperates with oncogenic genetic mutations to drive iCCA initiation and early growth. We have identified several experimentally validated Notch effector genes that could be targeted therapeutically in high-risk patients to limit the progression of pre-malignant disease into cancer.

Figure:


Notch-dependent malignant transformation of the biliary epithelium in iCCA formation
normal biliary epithelium
low/intermediate-grade dysplasia
high-grade dysplasia/ early iCCA
Proliferation
Notch ligands
+ tumour suppressor loss
+ hepatic injury
+ acquisition of ligand-rich niche
healthy cholangiocytes
mutant cholangiocytes (eg. loss of PTEN/p53)
NOTCH 2 receptor
NOTCH 2 ligands (JAGGED 1 or DLL1)
NOTCH 2 receptor
Notch ligand (JAGGED 1 or DLL1)
Cytoplasm
Nucleus
1 NOTCH 2 receptor activation in injured biliary epithelium
2 Proteolytic cleavage and nuclear translocation of the NOTCH 2-ICD
3 Overexpression of target genes (Hes1, HeyL, Nrarp, Cspg4, etc.)

P06-02
Safety and feasibility of Atezolizumab and Bevacizumab downstaging to liver transplantation of intermediate-advanced HCC: preliminary European experience on 7 patients

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Background and Aims: There is growing interest in the use of immune checkpoint inhibitors (ICIs) in tumor downstaging strategies. The combination of atezolizumab with bevacizumab (atezo+bev) is the only approved first-line systemic therapy for unresectable hepatocellular carcinoma (HCC) in Europe. We evaluated safety and feasibility of liver transplantation (LT) after atezo+bev HCC downstaging.

Method: An anonymized survey was sent to all centers affiliated to the European Society for Organ Transplantation (ESOT) between June and December 2022 inquiring about transplantation for HCC after systemic treatment with ICIs, with particular reference to downstaging with atezo+bev.

Results: In 6 out of 16 responding centers, 11 pts receiving ICIs (any kind) ahead of LT were collected. In 7 pts atezo+bev was given with a tumor downstaging intent. In all pts but one, other interventions (alone or in combination) were part of the downstaging strategy before atezo+bev. Main results are summarized in Table 1. At treatment inception, all pts were beyond Milan criteria (MC), 5 (71%) beyond up-to-7 (Up7) with a median AFP of 154 UI/ml (0.8 – 46679). After a median of 180 days on atezo+bev (90-660), 3 patients (43%) were within MC while 4 (57%) remained beyond Up7 with a median AFP at listing of 3 UI/ml (0.8 - 120). LT was performed after a median of 82 days (50-254) from last infusion and tumor necrosis at explant pathology was complete in 5 pts (71%) and partial in the remaining. Post-LT immunosuppressive regimen consisted of a combination of calcineurin inhibitors, mycophenolate and steroids in 72% of pts. There was no peri-operative mortality; 5 pts suffered post-LT major complications (2 biliary and 3 vascular) of which one leading to re-transplant. No early rejection was detected. Median follow-up was 227 days (80 – 631) with one patient experiencing tumor recurrence.

Conclusion: The combination of atezo+bev appears to be feasible in the HCC transplant setting. Observed increased morbidity may be related to selection bias of complex candidates due to previous intensive interventions. Further prospective studies are warranted in order to define, in pts with response to atezo+bev, the strength of the benefit LT confers upon systemic treatments.

Figure

Patients characteristics (=7 pts)	
Male (%) / Female (%)	6 (85%) / 1 (15%)
Age (median, range)	62 (58 – 72)
Underlying liver disease: - Viral Hepatitis - MAFLD	 5 (71%) 2 (29%)
Interventions before atezo+bev: - Surgery alone - Multiple ablation - Multiple TACE/TAE - TACE/TAE + surgery - TARE alone - No previous loco-regional therapies	 1 1 2 1 1 1
Tumor stage at inception of atezo+bev - Milan-In - Milan-Out , Up7-In - Up7-Out	 0 2 (29%) 5 (71%)
Tumor stage at LT (radiology) - Milan-In - Milan-Out, Up7-In - Up7-Out	 3 (43%) 0 4 (57%)
Tumor response at LT (explant pathology) - Complete necrosis - Partial necrosis	 5 (71%) 2 (29%)
Days on atezo+bev (median, range)	180 (90 – 660)
Days from last administration to LT (median, range)	82 (50 – 254)
Post-LT immunosuppression - CNI + mycophenolate + steroids - CNI + steroids - mTOR-i + mycophenolate	 5 (72%) 1 (14%) 1 (14%)
Post-LT complications - Vascular complications - arterial thrombosis - pulmonary embolism - Biliary complications - Early graft rejection	 3 (43%) 2 1 2 (29%) 0
Survival - Alive, tumor-free - Alive, with recurrence - Dead	 6 (86%) 1 (14%) 0

P06-04
Peripheral immune markers can detect hepatocellular carcinoma in blood in a Latin American cohort

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Background and Aims: Late detection of hepatocellular carcinoma (HCC) due to suboptimal surveillance with ultrasound is a major problem worldwide but with further importance in resource-limited settings. Blood biomarkers are urgently needed and currently alpha-fetoprotein (AFP) is the only accepted biomarker. However, AFP has poor accuracy for early HCC detection and has mainly been studied in resource-rich settings. We prospectively investigated circulating immune markers to detect HCC in 2 different groups of Latin American patients acting as discovery and validation cohorts.

Method: Through the ESCALON network we prospectively evaluated a discovery cohort of 127 individuals with HCC and 113 cirrhotic controls from 3 countries in Latin America (Argentina, Brazil and Ecuador) as well as a validation cohort of 145 HCCs and 75 cirrhotic individuals from a different set of institutions in Latin America (Chile, Peru, Argentina, Ecuador, Colombia). Blood samples were analyzed for 37 unique immune markers using the multiplex Bio-Rad platform. Differences between HCC and cirrhosis were analyzed via t-test and ANOVA, and tuned with lasso coefficient-bootstrap computing. We used leave-one-out cross-validation (LOOCV) to compute an ROC curve.

Results: In the discovery cohort 22 markers showed a significant difference between cases and controls for all size tumors and 15 for those tumors <5cm. A set of 5 markers which were highly differential in HCC vs cirrhosis controls identified via Lasso and bootstrap: HGF, MIP-3a, MIG, CCL-25, and MDC. The AUROC for this top-5 set in detecting HCC was 0.83 (CI 0.78-0.88) for all tumors and 0.75 (CI 0.66-0.83) for tumors <5cm. In this same cohort, the AUROC for AFP was only 0.69 for all tumors and 0.66 for tumors <5cm. The addition of AFP to the top-5 markers did not significantly increase the AUROC (0.83 to 0.85). We investigated the set of top-5 markers in the validation cohort and found that they could detect HCC with an AUROC of 0.73 (CI 0.642-0.810). The main differences between both cohorts were in the underlying liver diseases in HCC, with viral hepatitis being the most common in the validation cohort (42%) and non-alcoholic fatty liver disease in the validation cohort (56%).

Conclusion: Our study identified a set of 5 cytokines that can detect HCC by means of blood measurement in a discovery and validation cohort in Latin America. To our knowledge this is the first study assessing immune markers with a high degree of accuracy in a unique Latin American cohort.

P06-05-YI
Impact of the covid-19 pandemic on the treatment of resectable liver neoplasias: a cross-sectional study from Brazil

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Background and Aims: By 2030, 10 million patients needing surgical oncology will be from low and middle-income countries. Hepatocellular carcinoma is reported as the fifth most common cancer in men and the seventh in women worldwide. Surgical resection is the treatment option for patients with solitary hepatic tumors and very well-preserved liver function is fundamental for a better prognosis and outcome. The overload on health systems caused by the coronavirus pandemic has strongly affected Brazilian public healthcare (SUS), making the diagnosis of hepatocellular carcinoma more difficult due to lack of resources and professionals available for this. Under these circumstances, we aimed to evaluate the impact of COVID-19 on the surgical treatment-patterns for liver cancer performed by SUS during the peak of the COVID-19 pandemic.

Method: A retrospective and ecological study was conducted following the STROBE checklist. With data from DATASUS's Hospital Information System; An agency of the Brazilian Ministry of Health; patients' identities are preserved and conduction of any research with data from DATASUS is ethically approved. All hepatectomies from all hospitals were considered. Based on the PICO principle: People with liver cancer in Brazil from 2012 to 2021 (Population); hepatectomy (Interest); Brazilian region (Context), the study analyzes the absolute numbers of hepatectomies in oncology registered between January 2012 and December 2021. Statistical analysis was performed applying ANOVA one way and Tukey's test to assess the number of procedures compared over the years.

Results: The number of partial hepatectomies in oncology in Brazil, through the SUS, between January 2012 and December 2021, was 8,341 with a yearly average of 695 procedures. In 2020, the total number of hepatectomy cancer surgery suffered a decrease of 12,95% going from 1,019 procedures in 2019 to only 887 in 2020. A decrease of 23,80%, 36,73%, 34,54%, and 23,59%, in hepatectomy cancer surgery was reported in the months of May, August, October, and December respectively, when comparing 2019 and 2020. Despite vaccination starting in Brazil in 2021, we continue to see a decrease in the number of hepatectomies by 6,67%, compared to the number of Hepatectomies in 2019.

Conclusion: The decrease in the number of hepatectomies is apparent and may affect the prognosis of the patients who haven't had access to the health system during the covid 19 pandemic. However, secondary delays in diagnosis could affect our observation. To deliver safe, affordable, and timely cancer surgery to all, surgery must be at the heart of global and national cancer control planning and further research is needed to understand the impact of our observation in the outcome of patients.

Table

Year	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Surgeries	445	709	749	859	849	938	935	1019	887	951

P06-08-YI
Clinical and biologic qualification of the ABRS gene signature, a putative biomarker of benefit to atezolizumab and bevacizumab in advanced hepatocellular cancer

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Background and Aims: The Atezolizumab-Bevacizumab (AB) combination is a global standard of care in advanced Hepatocellular Carcinoma (HCC). However, prediction of response and survival benefit cannot be achieved in an individual patient. A recent study identified a 10-gene transcriptomic trait termed AB Response Signature (ABRS) (Zhu *et al* 2022) as a putative predictive biomarker of improved Overall Survival (OS) and Recurrence Free Survival (RFS). This finding lacks independent validation in untreated cohorts to differentiate prognostic from true predictive value.

Method: We evaluated the prevalence and clinicopathologic significance of ABRS+ in The Cancer Genome Atlas (TCGA) HCC samples (n=371) and validated ABRS as a predictor of OS and RFS. In addition, we made and analysed a bulk RNA sequencing (RNAseq) dataset from a cohort of resected HCC patients (n=57).
Differential Expression Analyses (DEA), Gene Set Enrichment Analyses (GSEA) and survival analyses were performed with R 4.2.2.

Results: The RNAseq patients' median age was 71.5 years (48.2-86.2), 84% were male and the main aetiology was Alcoholic Liver Disease (36%). Median OS was 81.4 months (95% Confidence Interval (CI) 55-NA) and the median RFS was 51.8 months (CI 41.9-NA).

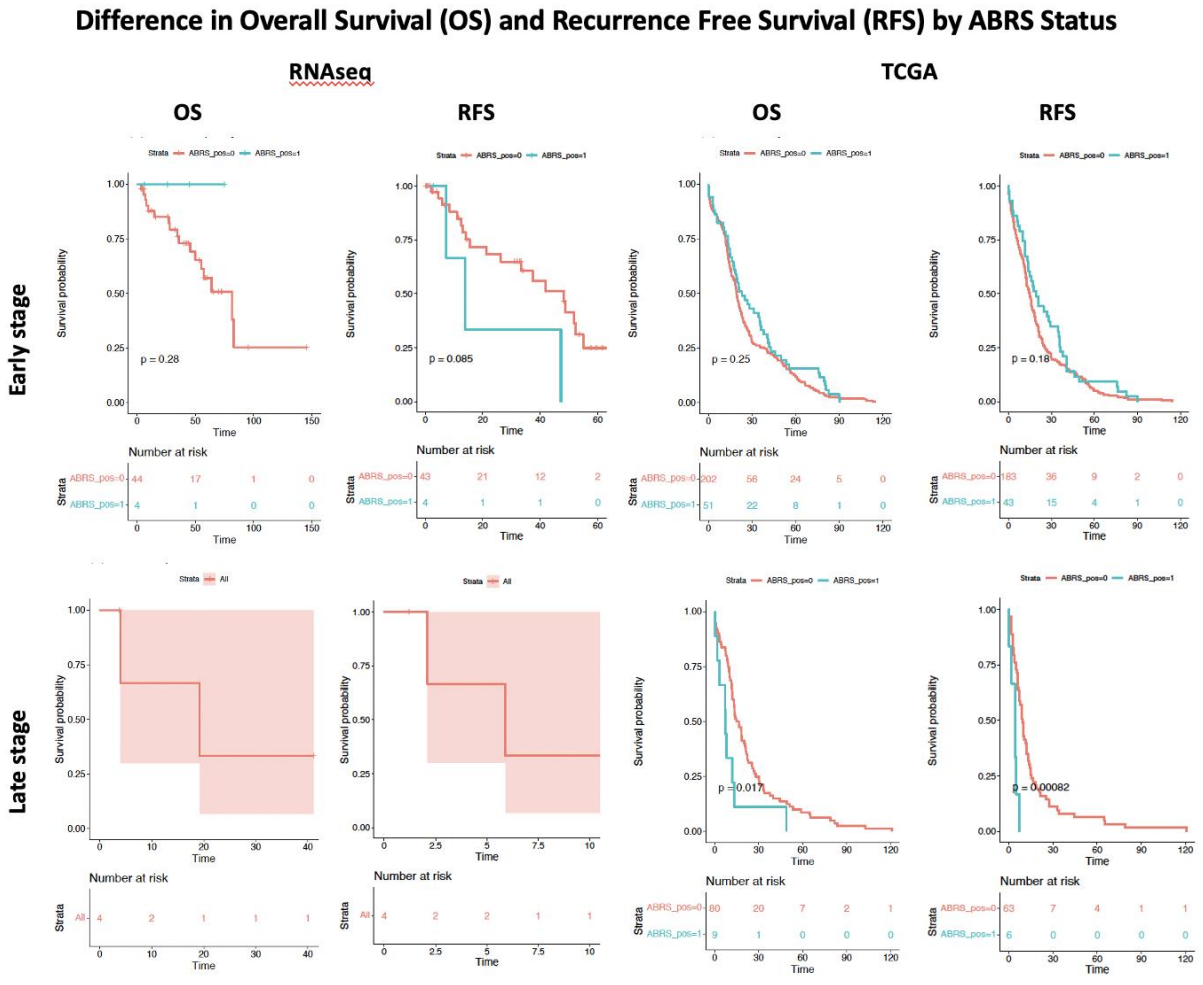
Individually only over-expression of TIMD4 was associated with shorter median OS of 19 months (CI 15.4-21.5, p=0.04) vs median OS of 22.7 months (CI 20.4-27). Over-expression of ABRS genes classified 68 patients as ABRS+ and 367 as ABRS-. DEA showed ICOS (p=0.01) and CTLA4 (p=0.01) were significantly more expressed in HCC vs background non-neoplastic liver. GSEA confirmed CTLA4's role with AIM2, FCRL3 and KLRC3 in immune regulation (p=0.0008) via GO:0002253 and GO:0045089.

ABRS did not significantly differ when comparing viral (n=41, 71%) vs non-viral (n=6) aetiologies. It was also not associated with age, sex, tumour size, Child-Pugh score, macrovascular or microvascular invasion.

TCGA ABRS+ patients with stage 3/4 disease (n=95, 24%) median OS was 7.1 months (CI 3.3-NA, n=85) which was significantly shorter than ABRS- (median OS = 16.4 months, CI 13.0-21, n=9, p=0.017) (Figure). Furthermore, their median RFS was 4.2 months (CI 1.8-NA) was significantly shorter than ABRS- median RFS of 9.1 months (CI 7-12, p=0.00082). There was no significant difference in median OS or RFS in early-stage (1/2) patients in TCGA or the RNAseq patients.

Conclusion: ABRS identifies a subset of patients with HCC characterised by evidence of a spontaneously immunogenic tumour microenvironment. ABRS did not cluster with salient clinicopathologic features of HCC including aetiology and was not prognostic in early-stage HCC patients. Lack of a strong prognostic role supports ABRS as a putative predictive marker for which validation in AB-exposed cohorts is warranted.

Figure:



P06-09
Correlation of single nucleotide polymorphisms and hepatocellular carcinoma in a Latin American population

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Background and Aims: Host genetics have shown to play an important role in addressing risk factors for developing HCC. Indeed, a family history of HCC is associated with a 2-fold increased risk of HCC. Several gene polymorphisms (SNP), including TLL1, MBOAT7 and STAT4 have been associated with HCC development. However, most of the studies addressing their risk association have been performed on Asian or European populations. In the current study, we investigate the association between these SNP and cirrhotic HCC in Latin Americans.

Method: DNA was isolated from blood samples from participants of the multi-center ESCALON project (www.escalon.eu). SNP analysis was performed using TaqMan-genotyping assay on 862 samples: 362 cirrhotic and 155 HCC patients from Argentina, Chile, Colombia, Ecuador, Peru, and 156 cirrhotic and 189 HCC from the Netherlands. Chi-Squared, Fisher's Exact test and multiple logistic regression were performed to evaluate the association between SNP and HCC.

Results: Median age of HCC cases was 68 y/o in Latin Americans and 67 y/o in Europeans, with 64% and 77% being male, respectively. The most common causes of HCC were NAFLD (46%) and alcohol use disorder (AUD, 25%) among Latin Americans, and AUD (35%) and NAFLD (19%) in Europeans. The proportion of Latin Americans expressing the risk allele for STAT4 (G) was 84% in HCC patients and 87% in cirrhotics, while among the Europeans the ratio was 93% in HCC and 97% in cirrhotics. The proportion of Latin Americans expressing the T risk allele for MBOAT7 was 21% for HCC and 16% for cirrhotic controls, while in Europeans the ratio was inverted to 14% in HCC and 21% in cirrhotics. The proportions of Latin Americans with HCC and a TLL1 pathogenic variant was 18% compared to 26% in cirrhotics without HCC. The calculated Odds-Ratio (OR) for HCC with the high risk STAT4 allele was 0.87 for Latin Americans (CI 0.51 - 1.53) and 0.48 for Europeans (CI 0.13 – 1.49), suggesting an inverse association risk, albeit not significance. For the MBOAT7 risk SNP the calculated OR was 1.16 (0.64 - 2.11) and 0.50 (0.16 - 1.60) for Latin Americans and Europeans, respectively, suggesting a non-significant increased risk for HCC in Latin Americans and reduced risk for Europeans. The TLL1 variant in Latin Americans showed a non-significant decreased odds for HCC (0.699 (CI 0.379 - 1.291). However, a subgroup analysis of HCV-associated HCC and TLL1 showed a non-significant increased risk of HCC (OR 2.07 (CI 0.93 - 4.59) in Latin Americans.

Conclusion: The results of this ongoing story clearly demonstrate that HCC risk gene variants show differential associations in Latin American and European populations. In particular, the MBOAT7 risk variant showed an inverted OR for HCC in Latin Americans compared to Europeans, clearly indicating

that one should be cautious in extrapolating risk profiles on basis of SNP data across continents. A larger study is ongoing to confirm these findings.

P06-10
Role of Etiology in Hepatocellular Carcinoma Patients Treated with Lenvatinib: A Counterfactual Event-Based Mediation Analysis

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Background and Aims: Whether the etiology of underlying liver disease represents a prognostic factor in patients with hepatocellular carcinoma (HCC) treated with lenvatinib is still a matter of debate. This study investigates whether the viral etiology of HCC plays a prognostic role in overall survival (OS).

Method: Data derived from a multicenter series of 313 HCC patients treated with lenvatinib between 2019 and 2022 were analyzed. Actuarial survival estimates were computed using the Kaplan–Meier method and compared with the log-rank test. We performed an event-based counterfactual mediation analysis to estimate direct (chronic inflammation and immunosuppression), indirect (tobacco smoking, alcohol use, illicit drug abuse with injections), and the total effect of viral etiology on OS. Results were expressed as hazard ratio (HR) and 95% CI.

Results: Median OS was 21 months (95% CI: 20–23) in the group with other etiologies and 15 months (14–16) in the group with viral etiology ($p < 0.0001$). The total effect of viral etiology was associated with OS (HR 2.76, 1.32–5.21), and it was mainly explained by the pure direct effect of viral etiology (HR 2.74, 1.15–4.45). By contrast, its total indirect effect was not associated with poorer survival (HR 1.05, 0.82–2.13). These results were confirmed when considering tobacco, alcohol consumption, or injection drug abuse as potential mediators. Median progression-free survival was 9 months (8–10) in patients with other etiologies and 6 months (5–7) in patients with viral etiology ($p < 0.0001$). No difference in terms of adverse event rate was observed between the two groups.

Conclusion: Patients affected by HCC with nonviral etiology treated with lenvatinib exhibit longer survival than those with viral etiology. This finding may have relevance in the treatment decision-making process.

P06-11
Prognostic performance of Toronto HCC risk index and the alpha-fetoprotein rate in patients with Hepatocellular carcinoma

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Background and Aims: Hepatocellular carcinoma (HCC) is a life-threatening complication of cirrhosis. The alpha fetoprotein (AFP) conventionally used for HHC screening and the Toronto HHC risk index (Toronto index) which is a simple score recently proposed for the prediction of HHC, could have prognostic value. Our objective was to assess the prognostic performance of the Toronto index and AFP rate at the time of diagnosis of HCC on the prediction of overall one-year survival.

Method: This is a retrospective study including consecutive cirrhotic patients with HCC followed in our department, between January 2010 and December 2019. Overall survival was assessed by Kaplan-Meier survival analysis using log-rank. Demographic, clinical, and paraclinical data were collected.

Results: A total of 219 cirrhotic patients were included. Sixty-one (27,8%) of them had HCC with a mean age of 64,3 ± 10,1 years and a sex ratio of 3,35. The patients were classified according to the BCLC classification: 3,2% stage (0), 33,8% stage (A), 28.9% stage (B), 19,1% stage (C) and 15 % stage (D). Toronto index was statistically associated with BCLC classification (p=0,011) but not with one-year survival (p=0,136). A significant correlation was noted between the AFP rate and the stage of the BCLC classification (p=0,04). Twenty-one patients (classified as stage BCLC 0 and A) underwent curative radiofrequency treatment (34,4%) and two patients underwent surgical resection (3,2%). Thirteen patients classified as stage B underwent chemoembolization (21,3%) and three patients treated with sorafenib (4,9%). One-year overall survival was 42,3% in patients with AFP below 14 ng/ml and 15,3% in patients with AFP above 14ng/ml (p = 0.032). The area under the ROC curve for the AFP rate in the one-year survival prediction was 0.664 [95% CI: 0.502-0.826].

Conclusion: At a cutoff of 14 ng/ml, the AFP rate had a good prognostic value in one-year survival prediction, unlike the Toronto index. This suggested the interest of its inclusion in the prognostic scores of the HCC.

P06-12
The association of glucagon-like peptide-1 receptor and poor prognosis of Northeastern Thai patients with cholangiocarcinoma

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Background and Aims: The high incidence of cholangiocarcinoma (CCA) has been noted in the Northeast of Thailand, where the high prevalence of diabetes mellitus (DM) is also high. Glucagon-like peptide-1 receptor (GLP-1R) agonist, an anti-diabetic medication, might be associated with the increased risk of CCA, but the association between GLP-1R and CCA has not been studied. This study, hence, aimed to reveal the possible roles of GLP-1R on the progression and prognosis of CCA in Thailand.

Method: GLP-1R expressions were determined in CCA tissues from Thai patients (N = 37) using immunohistochemistry. The association of GLP-1R and clinicopathological characteristics were analyzed by univariate analysis and Spearman's correlation using IBM SPSS ver 26.0. Expressions of GLP-1R in CCA cell lines were determined by Western blot analysis. The effects of GLP-1R agonist on CCA cell proliferation were examined by MTT assay.

Results: GLP-1R expression was not associated with the DM status of the patients. However, high GLP-1R expression was significantly associated with poorer histological grading ($P < 0.05$). In addition, GLP-1R expressions were also correlated with higher TNM staging (The 8th Edition AJCC staging for Hepato-pancreato-biliary cancer) in perihilar subtype (Spearman's rho = 0.560, $P < 0.05$). All tested CCA cell lines expressed GLP-1R, but the treatments of CCA cells with GLP-1R agonists exhibited minimal effects on cell growth.

Conclusion: GLP-1R expressions are associated with poor histological grading of CCA and poor prognosis of patients with perihilar subtype. The effects of GLP-1R agonists on CCA progression need to be further investigated.

P06-13
Synergistic Anticancer Effect of Immunotherapy and PI3Kγ Inhibition Combination Therapy in Liver Cancer

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Background and Aims: The immune checkpoint inhibitors (ICIs)-based combination therapies in hepatocellular carcinoma (HCC) patients are very effective but not for all patients. Suppressive myeloid cells mediate resistance to immunotherapy. PI3Kγ inhibition can target suppressive macrophages and enhance efficacy of ICIs. However, how PI3Kγ inhibitors function in HCC tumor microenvironments (TME) to activate specific immune cells is underexplored.

Method: The effect of the novel PI3Kγ inhibitor, AZD3458, was assessed in murine (BNL and RIL-175) HCC cell lines and BNL syngeneic model. We used PI3Kγ inhibitor (AZD3458; 30 mg/kg administered orally, daily), TKI (lenvatinib; 10 mg/kg administered orally, daily) and ICI (anti-PD-1 mAb; 10 mg/kg intraperitoneally, daily). The antitumor effects of PI3Kγ inhibitor were also tested *in vitro* using human HCC cell lines (Huh7 and Hep3B) and human peripheral blood mononuclear cells (PBMCs).

Results: AZD3458 enhanced antitumor activity of the combinational treatment of ICI and TKI in BNL, RIL-175, and BNL syngeneic models, increasing NK cell activation status. Immune and TME biomarker analysis of BNL tumors revealed that AZD3458 monotherapy or the combinational treatment of ICI and TKI activated NK cells by repolarizing the M2 phenotype of tumor-associated macrophage (TAM) cells. Co-treatment of AZD3458 enhanced the cytotoxicity of the combinational treatment of ICI and TKI against human HCC cell lines and human PBMCs *in vitro*. AZD3458 increased the expression of activation cytokines for NK cells (IL-1β, IL-12, IL-23, CCL2, and TNF-α) and decreased the expression of inhibitory cytokines for NK cells (IL-6 and IL-10) in human PBMC-derived M2-polarized macrophages. Furthermore, the inhibitor both increased and decreased the expression levels of the M1 macrophage marker iNOS and the M2 macrophage marker Arg1, respectively.

Conclusion: The results indicate that the PI3Kγ inhibitor specifically targets TAMs and alters their function to achieve anti-tumor effects. The findings of the study suggest that inhibiting PI3Kγ could be a crucial part of the combined anti-tumor treatment using ICI and TKI in HCC.

Figure:

Figure 1. Kaplan-Meier overall survival analysis of HCC patients stratified by PIK3CG expression level and IHC staining of TAMs in TMA slides according to the grade of HCC.

Figure2. PI3Kγ is a marker of human HCC-associated Mφs. Figure 3. Selective cytotoxicity of PI3Kγ inhibitor to Mφs. Figure 4. PI3Kγ inhibitor reprogram M2-polarized Mφs. Figure 5. PI3K inhibitor restores NK cells-mediated cytotoxicity to HCC cells inhibited with M2-polarized Mφs. Figure 6. PI3Kγ is a marker of mouse HCC-associated Mφs. Figure 7. PI3Kγ inhibitor and Immunotherapy combination therapy reduce tumor progression in the HCC subcutaneous model. Figure 8. PI3Kγ inhibitor and Immunotherapy combination therapy reduce tumor progression in the HCC Orthotopic model.

P06-14
The role of radiomics in the diagnostic and therapeutic path of hepatocellular carcinoma treated with trans arterial radio embolization

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Background and Aims: Hepatocellular carcinoma (HCC) is the fifth neoplasia worldwide. Trans-arterial radioembolization (TARE) is a locoregional therapy used in case of advanced disease. A growing body of evidence is implementing Radiomics features applied to radiological imaging to extract quantitative information regarding HCC and its surrounding environment. Accordingly, we aimed to: (**I**) asses biochemical, clinic and radiomic related features pre-/post TARE treatment; (**II**) evaluate radiomics variations that correlate with response to treatment (evaluated with mRECIST criteria) and overall survival (OS) (tables 1-2).

Method: Patients with advanced HCC treated with TARE across a range of 10 years were retrospectively reviewed. Radiomic analysis was performed, using the LIFEx program, on the pre- and 3 months post-TARE CT arterial phase. Clinical-laboratory and radiomic-CT features were then analyzed to explore pre- vs. post-treatment variation using descriptive statistics by the Fisher's exact test and T- test.

Results: In total, n=20 pre-TARE and n=14 post-TARE were included. After TARE a worsening of liver function is common, as shown by the increase in the Child-Pugh score ($p = 0.0001$). Out of the radiomics parameters assessed, intensity, morphology, GLCM, GLRLM and GLSZM were significantly modified at post-TARE assessment ($p<0.0001$). Notably, the spectrum of specific radiomic intensities and GLCM feature demonstrated similar trend when compared with mRECIST criteria predicting response to therapy both within pre- and post-TARE sub-groups. Similarly, some of the intensity and GLCM radiomics features were found to be associated with improved survival within the pre-TARE patient ($p=0.029$). The same for survival was true in the post-TARE cohort when intensity, GLCM, GLRLM and GLSZM were analyzed.

Conclusion: Our study suggests Radiomics CT-based analysis during both pre- and post-TARE for HCC might be a promising non-invasive tool able to provide comparable results to the well-established mRECIST criteria for the prediction to response to therapy and estimation of later overall survival. Future prospective longitudinal cohort analysis will need to corroborate our preliminary findings.

Tables:

1.Radiomic features before TARE which correlate with OS post-TARE (mean ± SD): 8.46 ± 11.60 months.

Radiomic features	P value
INTENSITY BASED Total Calcium Score IBSI No	0.0328
GLCM_Autocorrelation IBSI QWB0	0.0285

2.Radiomic features after TARE which correlate with OS post-TARE (mean ± SD): 8.46 ± 11.60 months.

Radiomic features	P value
INTENSITY_BASED MaximumGreyLevel HU BSI 84IY	0.0445
INTENSITY BASED MedianAbsoluteDeviation HU IBSI N72L	0.0439
INTENSITY BASED AreaUnderCurveCsh HU IBSI No	0.0000
GLCM Contrast IBSI ACUI	0.0025
GLRLM LowGreyLevelRunEmphasis IBSI V3SW	0.0117
GLRLM_HighGreyLevelRunEmphasis IBSI G3QZ	0.0221
GLSZM LargeZoneEmphasis IBSI 48P8	0.0488

P06-18
Predictors of extrahepatic recurrence after transarterial chemoembolization as first-line therapy for hepatocellular carcinoma

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Background and Aims: The literature regarding the risk of progression to extrahepatic disease and clinical factors associated with the development of metastases in patients with hepatocellular carcinoma (HCC) treated with transarterial chemoembolization (TACE) is sparse. We aimed to assess the incidence of extrahepatic recurrence and to identify clinically relevant risk factors for the development of metastases in patients with HCC treated with TACE as first-line treatment.

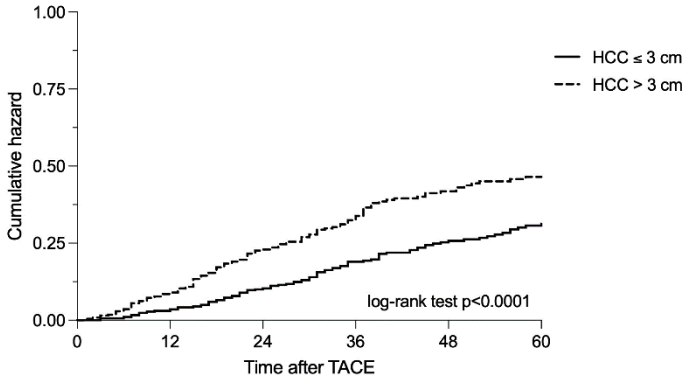
Method: From the Italian Liver Cancer (ITA.LI.CA) database, data of 981 HCC patients undergoing TACE as first-line treatment were retrieved and retrospectively analyzed. Incidence of extrahepatic recurrence was compared between two groups according to the diameter of the largest liver lesion at the time of TACE (HCC ≤ 3 cm vs. HCC > 3 cm). Multivariate Cox regression was used to identify predictor of extrahepatic recurrence.

Results: During a median follow-up of 27.0 months (IQR, 13.2-49.0) the overall recurrence rate was 75.4%. Only 78/981 patients (8.0%) had an extrahepatic tumor localization at first recurrence (5.4% in the ≤3 cm group and 10.7% in the >3 group; p=0.002), while the overall extrahepatic recurrence rate was 26.0% (21.2% and 31.0% patients in the ≤3 cm and >3 cm groups, respectively; p=0.0006) (Figure 1). Compared to those with larger tumors, patients with HCC ≤ 3 cm had a significantly longer recurrence-free survival (12.0 [95% CI 10.7-13.3] vs. 9.7 [95% CI 8.2-11.2] months; p=0.02) and overall survival (52.5 [95% CI 45.5-59.4] vs. 34.7 [95% CI 30.7-38.7] months; p<0.0001). HCC size ≥ 3 cm, multifocality and AFP levels were independent predictors of extrahepatic recurrence.

Conclusion: Although the majority of patients treated with TACE do not develop metastases during their lifespan, knowledge of risk factors for extrahepatic recurrence (HCC size, multifocality, AFP levels) may help to assess patient prognosis and to identify patients deserving closer follow-up in order to start early systemic therapy.

Figure:

Figure 1. Cumulative hazard of extrahepatic recurrence among patients with HCC ≤ 3 cm and > 3 cm treated with first-line TACE.



N° at risk						
HCC ≤ 3 cm	504	412	316	231	161	108
HCC > 3 cm	477	351	227	149	95	72

P06-20
Factors associated with recurrence and survival in liver transplant patients with HCC - a single center retrospective study

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Background and Aims: Hepatocellular carcinoma is the most common primary tumor of the liver and is diagnosed in more than a half million people worldwide each year. This study aims to assess factors associated with the recurrence and survival of patients with hepatocellular carcinoma and liver transplantation in a cohort of patients.

Method: This was a descriptive retrospective study of liver transplant patients from the in King Faisal Specialist Hospital & Research Center during the period January 2010- January 2022. Demographic, clinical, imaging, and pathology variables were analyzed.

Results: 295 liver transplants were performed during the study period, 20 cases (6.8%) had one or more hepatocellular carcinomas in the explant, and 60% of these patients were men, mean age 61 year (31-73), 15 patients received liver transplant from living related liver donors

All patients with HCC recurrence included were cirrhotic at the time of HCC diagnosis pre transplant, with different aetiologies, but most of them were due to NASH (50%), followed by hepatitis C virus infection (45 %), and hepatitis B virus infection (5%). With regards AFP pre transplant, it was less than 100 in 19 patients and 1 patient had AFP level at 450. 12 out of 20 patients had locoregional therapy (LRtx) pre transplant (RFA in 6 patients, 3 TACE and TARE for 3 patients).

In the explant liver pathology specimen, 5% had more than 3 focus, (13 patients) and in 16 patients HCC lesions were out Milan . 2 patients were diagnosed with combined HCC Cholangiocarcinoma on explant liver (cHCC-CC). Both had low AFP level pre Ltx and at the time of HCC recurrence and none had locoregional therapy pre Ltx and both were outside Milan with microvascular invasion in one of them. Both died 2 years after Ltx due to disease.

HCC recurrence was diagnosed with CT scan in 14 patients, MRI scan in 6 patients, and percutaneous liver biopsies in 16 patients. With regards to AFP level at the time of HCC recurrence, all patients had AFP level below 100 expect 2 patients (122, and 5549) respectively at the time of HCC recurrence
Hepatic HCC recurrent was in 5 patient, liver with extra hepatic metastasis in 15 patients (lung in 10 patient pelvic and bone in 4 patient and chest wall involvement in 1 patients)

During follow-up, 2.3% of the patients died in an average time of 84 months. The 1, 3 and 5-year survival rates were 100%, 95% and 85%. Recurrence of HCC was the cause of death in 2.3% of patients.

Conclusion: The HCC recurrence rate post transplantation was relatively low. The recipients of DDLT & LDLT had similar recurrence rates and overall survival. The factors associated with these outcomes were vascular , lymphocytic invasion, moderate tumor differentiation. AFP was also useless in the diagnosis of HCC and or recurrent HCC

P07-01
Efficacy of atezolizumab in combination with bevacizumab in patients with unresectable hepatocellular carcinoma not previously treated with systemic therapy: efficacy results from the interim analysis of the Phase IIIb Italian AMETHISTA trial

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Background and Aims: In patients with unresectable and previously untreated hepatocellular carcinoma (HCC), the IMbrave150 trial showed statistically significant and clinically meaningful benefit in overall survival (OS) and progression-free survival (PFS) in atezolizumab plus bevacizumab arm compared to sorafenib arm. The objective of the single-arm, phase IIIb, AMETHISTA trial was to assess the safety and efficacy profile of atezo plus bev in an Italian population of patients selected according to IMbrave150 criteria. An interim analysis of efficacy (secondary endpoints) was conducted at approximately one year from the end of enrolment.

Method: Patients had histologically confirmed HCC not amenable to surgery and/or locoregional therapies and not treated with previous systemic therapy, at least one measurable lesion, ECOG PS 0-1, Child Pugh class A. Atezo 1200 mg and bev 15 mg/kg were given IV Q3W until unacceptable toxicity or loss of clinical benefit. Secondary efficacy endpoints were OS, PFS, time-to-progression (TPP), objective response rate (ORR), duration of response (DoR) and post-progression survival (PPS).

Results: 152 patients were enrolled and 149 (118 males, median age 69 years) were treated. Etiology (potentially multiple) was hepatitis B in 32 patients (21.5%), hepatitis C in 64 (43.0%), alcohol in 39 (26.2%) and non-alcoholic liver damage in 21 (14.1%). Most patients had ECOG PS 0 (126 patients, 84.6%) and BCLC stage C (116 patients, 77.9%). Varices were present in 32 (21.5%) patients. 54 patients (36.2%) had received prior loco-regional therapy. At a median follow-up of 13.4 months, 50 patients (32.9%) were still on treatment and 99 (65.1%) had discontinued treatment (mainly due to disease progression, 57 patients, 57.6% of those who had discontinued). At the database lock for the interim analysis, 50 patients (33.6%) had died and 48 (31.6%) had discontinued follow-up (due to death in 38 of them, 79.2%) while 36 (23.7%) were still in follow-up. The median exposure to atezo and bev was 8.3 and 7.3 months (12.0 and 11.0 cycles), respectively. Median OS was 18.23 months (95% CI, 15.38-not estimable [NE]), median PFS was 8.51 months (95% CI, 7.52-11.24), median TTP was 10.84 months (95% CI, 8.18-15.74) and median PPS was 9.1 months (95% CI, 7.26-13.80). Best response to treatment was as follows: 41 patients (26.9%) had objective response, 3 (1.9%) complete response and 38 (25%) partial response; 84 patients (55.3%) had stable disease and 16 (10.5%) had progressive disease (PD; response was unknown in 11 patients). At the cut-off date, 73 patients (48.0%) had PD. In patients with response, the median DoR was NE due to the still too low number of patients with progression or death.

Conclusion: This interim analysis of the AMETHISTA trial confirms in an Italian population the efficacy previously reported in the international IMbrave150 trial at a corresponding follow-up period.

P07-07
Metformin and statin reduce the risk of hepatocellular carcinoma among chronic hepatitis C patients failed to antiviral therapy

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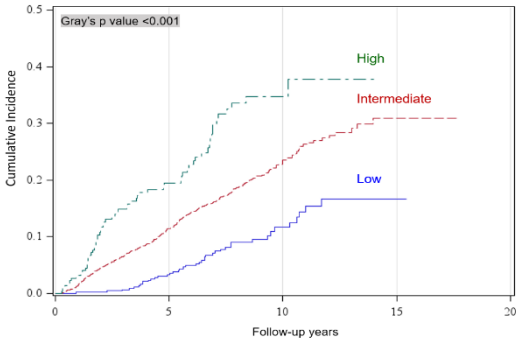
Background and Aims: Chronic hepatitis C (CHC) patients who failed to antiviral therapy are at high risk of hepatocellular carcinoma (HCC), especially among patients with diabetes mellitus (DM). We aimed to evaluate whether metformin or/and statin reduces HCC risk among diabetic and/or hyperlipidemic CHC patients who failed to achieve a sustained virological response (SVR) after antiviral therapy.

Method: CHC patients who failed to interferon-based therapy were enrolled in a large-scale, multicentre cohort in Taiwan (T-COACH). HCC 1.5-year after interferon-based therapy was identified by linking to the cancer registry databases from January 2003 to December 2019. After considering death as a competing risk, the cox subdistribution hazards for HCC development before or after adjustments were used.

Results: Of 2,779 CHC interferon-failed patients, 480 (17.3%) developed new-onset HCC and 238 (8.6%) died. DM non-metformin users had a higher proportion of HCC risk than DM metformin users or non-DM patients [23.6% vs. 17.2% or 16.4%] with an adjusted hazard ratio (aHR) (95% CI) of 1.53 (1.15-2.04, p=0.003, compared with non-DM patients). In contrast, hyperlipidemia (HLP) statin users had a lower proportion of HCC risk than HLP non-statin users or non-HLP patients [9.4% vs. 13.2% or 19.3%] with an aHR (95% CI) of 0.51 (0.38-0.69, p<0.001, compared with non-HLP patients). According to the aHR, the risk score of DM non-metformin users was estimated at 1.5 when other comparators were at 1. In contrast, HLP statin users was estimated at 0.5 when other comparators were at 1. Recombined risk score by the two risk factors was stratified as high risk, intermediate risk and low risk. The 5-year cumulative incident of HCC among non-SVR patients with low risk, intermediate risk and high risk was 3.4%, 11.5% and 19.5%. The aHR (95% CI) of HCC was 1.80 (1.31-2.47) in intermediate-risk patients and 2.85 (1.90-4.29) in high-risk patients when compared to low-risk patients as **Figure**.

Conclusion: Among non-SVR CHC patients, DM patients with metformin use or HLP patients with statin use greatly decreased the risk of HCC after antiviral therapy. A simple risk score contributed by DM non-metformin use of high risk, and HLP statin use of low risk could predict the new-onset HCC of CHC patients with non-SVR.

Figure:



Risk level	At risk No.	Cumulative Incidence (%) 1Y	3Y	5Y	8Y	10Y	Crude HR (95% CI) P value	*Adjusted HR (95% CI) P value
Low	434	0.2	0.7	3.4	9.0	11.7	1	1
Intermediate	2,123	1.5	6.6	11.5	18.7	23.6	2.25 (1.64-3.09) P<0.001	1.80 (1.31-2.47) P<0.001
High	222	3.2	14.9	19.5	33.6	34.8	4.06 (2.74-6.01) P<0.001	2.85 (1.90-4.29) P<0.001

*Adjusted for Age, Gender, AST, ALT, Liver cirrhosis, Aspirin, and NSAIDs

P07-08
The Differences in Profiles of Intratumoral Microbiota in Cholangiocarcinoma and Hepatocellular Carcinoma are Observed

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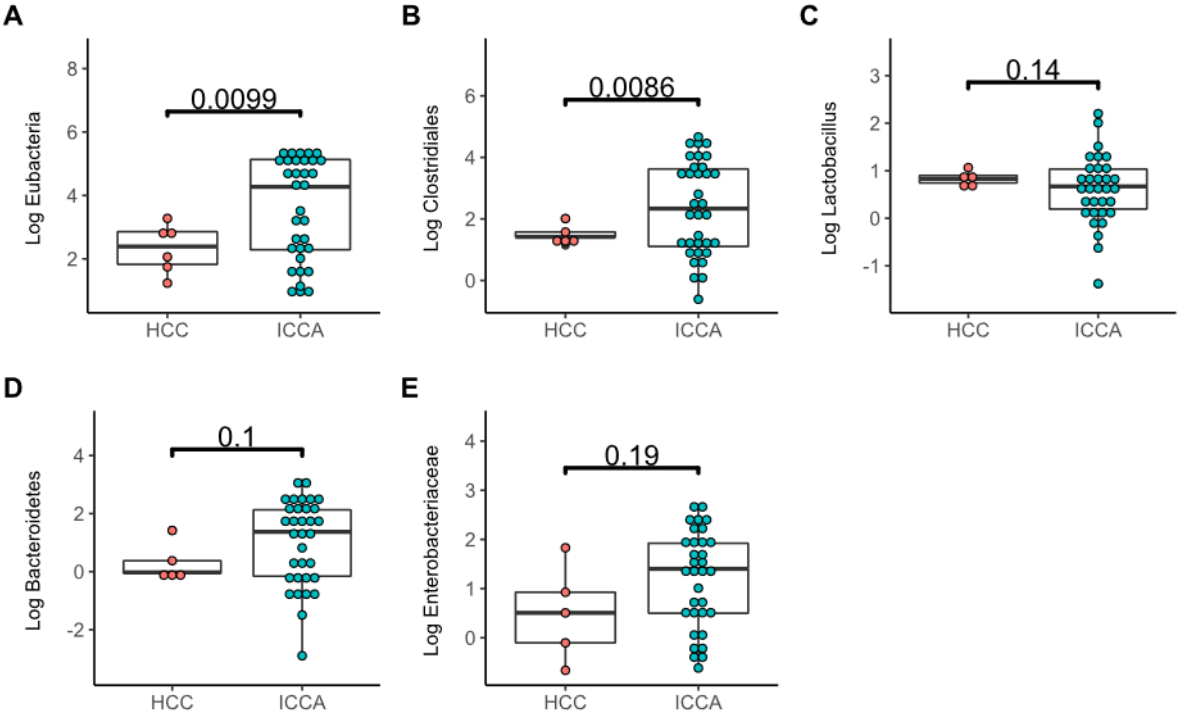
Background and Aims: Cholangiocarcinoma (CCA) is not a common cancer in the world population, however the incidence of CCA in the northeast of Thailand is up to 80 cases per 100,000 population per year. Although gut microbiota in CCA has been investigated, the profiles of intratumoral microbiota in CCA have been rarely determined. CCA has shown the highest biodiversity in gut microbiota profiles, when compared to hepatocellular carcinoma (HCC), but no comparative study of intratumoral microbiota between CCA and HCC has been determined. The present study aimed to compare the difference in profiles of intratumoral microbiota between CCA and HCC.

Method: Thirty-one patients with liver mass which clinically suspected intrahepatic cholangiocarcinoma (ICCA) or HCC planning for biopsy or surgery were recruited in the study. Bacterial genomic DNA samples were extracted from the tumors using a commercial genomic DNA isolation kit (Qiagen, Hilden, Germany). The population of the bacterial gut microbiota in Phyla of Firmucutes (Clostridales and Lactobacillus acidophilus), Bacteroidetes, and Enterobacteriaceae were observed. Those bacteria were determined and calculated by real-time qPCR process.

Results: 25 of 31 patients were diagnosed with ICCA and 6 were HCC. The median age of patients with ICCA and HCC was 65 (60,69) and 64.5 (60.69) years old, respectively. Amount of Eubacteria was significantly greater in ICCA than that of HCC (p=0.009, Figure 1). Interestingly, Clostridiales was found elevated in ICCA, when compared to that in HCC, (p=0.0086, Figure 1). However, other phyla and F/B ratio were not different between two groups. Since our study was done in a small sample size, the large number of patients with the next generation sequencing should be used to further investigate the profiles of intratumoral microbiota in both ICCA and HCC in the future.

Conclusion: These findings suggest that the profiles of intratumoral microbiota between ICCA and HCC are differences, as indicated by increased Eubacteria and Clostridiales in ICCA.

Figure 1:


A
Log Eubacteria
0.0099
HCC
ICCA
B
Log Clostridiales
0.0086
HCC
ICCA
C
Log Lactobacillus
0.14
HCC
ICCA
D
Log Bacteroidetes
0.1
HCC
ICCA
E
Log Enterobacteriaceae
0.19
HCC
ICCA

P07-09-YI
Hepatocellular Carcinoma in HIV-infected patients: clinical presentation and outcomes

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Background and Aims: As life expectancy for people living with HIV (PLWH) improves, hepatocellular carcinoma (HCC) has become a non-AIDS complication with a high impact on morbidity and mortality of PLWH. We sought to compare outcomes in PLWH versus non-HIV-infected patients with cirrhosis treated for HCC in three hospitals in Lombardy, Italy.

Method: A retrospective analysis of prospectively followed patients diagnosed with HCC from 01/06/2006 to 15/04/2022 was performed. Firstly, the clinical characteristics, access to treatment and survival of HIV-patients with HCC were described. Secondly, differences in characteristics among HIV and non-HIV subjects were assessed. Propensity score (PS) to address potential confounders due to unbalanced distribution of baseline characteristics of PLWH HCC patient and associations between HIV status, and CD4-count and survival will be calculated.

Results: We identified 65 HIV patients with cirrhosis and first diagnosis of HCC (median age 54 [44-73] years, 92% males, 63% HCV-pos, 73% Child-Pugh A, median MELD 10 [6-26], 68% no esophago-gastric varices, median CD4+ count 405 [44-1701] cells/mm^3, 12.5% with a previous AIDS diagnosis, median nadir CD4+ count 164.5 [11-750] cells/mm^3) and 464 non-HIV patients (median age 68 [33-89] years, 75% male, 77% Child-Pugh A, median MELD 9 [6-33], 64% no esophago-gastric varices). In PLHW, HCC was single nodule in 55%, median size was 2.5 cm (1.0-6.3), 57% "Milan-in". BCLC stages were 55% 0/A, 13% B, 21% C and 11% D. 31 (50%) patients received first-line curative treatment. In non-HIV patients, HCC were single nodule in 57%, median size was 2.6 cm (0.5-18), 66% "Milan in". BCLC stages were 68% 0/A, 17% B, 14% C and 1% D; 284 (61%) patients received a first line curative treatment (Table 1). Median follow up was 47 (0.2-175) months for PLWH and 30 (0.5-199) for non-HIV patients. For PLWH the 5-year overall survival rate was 58% compared to 56% for non-HIV subjects (log rank p =0.61).

Conclusion: HCC patients with HIV were more frequently male, younger, with multiple etiologies, poorer liver function and worse HCC stage at presentation than patients without HIV. Nevertheless, PLWH have similar prognosis and access to HCC treatments as non-HIV patients.

Table:

Variable		HIV N=65	Non-HIV N=464	p-value
Age, years*	66 (33-89)	54 (44-73)	68 (33-89)	<0.001
Sex	407 (77%)	60 (92%)	346 (75%)	0.002
Etiology				<0.001
HCV	324 (61%)	41 (63%)	283 (61%)	
HBV	49 (9%)	2 (3%)	47 (10%)	
HCV+HBV	29 (6%)	17 (26%)	12 (2.5%)	
HDV	17 (3%)	5 (8%)	12 (2.5%)	
Non viral	110 (21%)	0	110 (24%)	
CPT				<0.001
A	380 (76%)	41 (73%)	339 (77%)	
B	107 (22%)	10 (18%)	97 (22%)	
C	10 (2%)	5 (9%)	5 (1%)	
CPT A vs other	380 (76%)	41 (73%)	339 (77%)	0.54
MELD	9 (6-33)	10 (6-26)	9 (6-33)	0.11
Varices	183 (36%)	18 (32%)	165 (36%)	0.57
BCLC				<0.001

P07-10
Hepatocellular carcinoma (HCC) management in a tertiary HPB centre - lessons from the southeast coast of the UK

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Background and Aims: Europe is facing an epidemic of liver disease. The number of new cases and deaths from liver cancer could rise by >50% by 2040 and is already among the top 3 causes of cancer death in 46 countries (1). Although a variety of treatment options are now available to patients, response rates are variable. Royal Surrey NHS Foundation Trust is a regional HPB/HCC centre in the UK, with a catchment area of ~1.3 million. Our aim was to undertake a service evaluation and examine clinical outcomes.

Method: We reviewed the bi-monthly HCC Multi-disciplinary Meeting reports and patient records at the Royal Surrey County Foundation Trust. We analyzed the demographics of the patients, aetiology, prognostic scores, treatments received and, where possible, the survival statistics of patients discussed between January 2020 & December 2022.

Results: 149 patients were discussed in 421 discussions , with an average of 2.83 discussions per patient. Male to female ratio was 3.3:1. Aetiology of those diagnosed found 32.2% due to Non-alcoholic Fatty Liver Disease (NAFLD)/Non-Alcoholic Steatohepatitis (NASH), 27.5% due to alcohol liver disease (ALD), 24.8% cryptogenic, 9.4% due to viral hepatitis B or C, 4% due to autoimmune liver disease and 2.7% from Haemochromatosis or Alpha-1-Antitrypsin deficiency. Child-Pugh Scores were available for 97 patients i.e. A=72, B=22, C=3. Furthermore, ALBI scores of 58 patients were calculated i.e., Grade 1=28 patients, Grade 2=27 patients and Grade 3=3. Alpha-fetoprotein (AFP) was negative in 55% of the patients. Patient numbers who received TACE/TAE, ablation, resection, transplantation, systemic therapy, SIRT and best supportive care, were 54, 20, 11, 1, 19 ,15 and 19 respectively.. An average of 3.02 TACE procedures were performed per patient. Ten patients had histological confirmation before receiving treatment. The mean time to progression of disease was 7.86months. Most patients have stable disease and remain under surveillance.

Conclusion: Our results demonstrate a rising incidence of disease over the years studied. NAFLD/NASH has overtaken ALD as the underlying aetiology for HCC. More than half of our patients are AFP non-secretors. Despite increased treatment options and regular surveillance, time of progression of disease was quite low for some patients.

References:
(1)Global Burden of primary liver cancer in 2020 & Predictions to 2040 – Dec 2022
Journal of Heptatology, EASL,
Harriet Rumgay, Melina Arnold, Jacques Ferlay, .,

P07-11
Regorafenib in patients with unresectable hepatocellular carcinoma in real-world practice in the European Union: Final analysis of the prospective, observational REFINE study

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Background and Aims: In the final analysis of patients (pts) with unresectable hepatocellular carcinoma (uHCC) from the observational REFINE study of regorafenib (NCT03289273), treatment-emergent adverse events (TEAEs) were consistent with those reported in the global, phase 3 RESORCE trial (Kim YJ, ILCA 2022). Median overall survival (OS) in the global cohort was 13.2 months. Here, we present the final analysis of pts with uHCC from European Union (EU) countries in REFINE.

Method: REFINE was an international, prospective, multicenter study that enrolled pts with uHCC for whom the decision to treat with regorafenib was made by their physician before enrollment, according to the local health authority approved label. The primary aim was safety, including incidence of TEAEs (MedDRA v25) and dose modifications due to TEAEs. Secondary endpoints included OS, progression-free survival, and treatment duration.

Results: Of the 1005 evaluable pts, 357 (36%) were from EU countries (France [14%], Austria [5%], Italy [5%], Greece [4%], Netherlands [3%], Spain [3%], Belgium [1%], Denmark [1%], and Sweden [< 1%]). At baseline, median age of EU pts was 68 years (range 30–90), and 87% were male; the most common HCC etiology in EU pts was alcohol use (39%) vs hepatitis B in the global cohort (38%; **Table**). Transarterial chemoembolization (TACE) was the most common previous non-systemic treatment received by EU pts (43% vs 58% [global cohort]). The initial daily regorafenib dose was 160/120/80/40 mg in 45%/7%/44%/4% of EU pts and 47%/11%/40%/3% of all pts. Median treatment duration was 3.7 months (range < 0.1–38.9) in EU pts and in all pts. Of the most common TEAEs (EU pts), diarrhea and asthenia incidences were higher in EU pts vs all pts (38% vs 29% and 26% vs 11%, respectively), whereas hand–foot skin reaction was less common (25% vs 33%). TEAEs leading to dose modifications were comparable in EU pts vs all pts (48% vs 45%). Median OS from start of regorafenib in EU pts (11.1 months; 95% CI 9.4, 12.9) was comparable with the global cohort. Median OS was 14.6 months (95% CI 11.6, 16.6) in Child–Pugh A EU pts.

Conclusion: Final data from the global, real-world REFINE study confirm the safety and effectiveness of regorafenib in EU pts with uHCC in keeping with the findings from the global cohort.

Figure:

n (%)	**EU pts (n = 357)**	**All pts (N = 1005)**
Eastern Cooperative Oncology Group performance status[a]		
0/1	285 (80)	829 (82)
≥2	20 (6)	60 (6)
Child–Pugh class[a]		
A	209 (59)	618 (61)
B	58 (16)	123 (12)
C	2 (1)	5 (<1)
Barcelona Clinic Liver Cancer stage[a]		
B	53 (15)	133 (13)
C	227 (64)	625 (62)
Other	9 (3)	34 (3)
HCC etiology (multiple responses)[a,b]		
Alcohol use	139 (39)	250 (25)
Hepatitis C virus	81 (23)	242 (24)
Nonalcoholic steatohepatitis	48 (13)	66 (7)
Hepatitis B virus	37 (10)	382 (38)
Metastases at baseline	178 (50)	591 (59)
Vascular invasion at baseline	119 (33)	346 (34)

[a]Missing/unknown or non-evaluable data not shown.
[b]Excluding genetic/metabolic and other.

P07-12
Survival in people living with HIV with or without recurrence of hepatocellular carcinoma after invasive therapy

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Background and Aims: to address the overall survival (OS) and recurrence (RE) in people living with HIV (PLWH) treated with invasive therapy (IT) for hepatocellular carcinoma (HCC).

Method: this is a retrospective cohort study on PLWH with HCC diagnosed between 2000-2021. The study outcomes were OS and RE, investigated by use of Kaplan-Meier curves. The Cox proportional hazard regression model was used for multivariate analyses.

Results: the analysis included 41 PLWH with HCC who underwent invasive therapy [liver resection (LR, N=6) orthotopic liver transplantation (OLT, N=11) radiofrequency thermo-ablation (RFTA N=6) chemo/radioembolization (CRE, N=18)]. Characteristics of participants are summarized in Table 1.
At diagnosis of HCC differences between participants who survived and those who died were found in relation to median number of nodules >3 p=0.01, alpha fetoprotein (AFP) levels, p=0.015 as wells as AFP =>28 ng/mL vs. <28ng/mL, p=0.002 and CD8 cells count, p=0.020. OLT recipients had the best prognosis respect to those who received other invasive therapy, p=0.0038.
Recurrence was observed in 19/41 (46.3%) PLWH; in 36.7% (IQR 23.1% – 55.0%) of participants at 2 years and in 52% (IQR 35.7% – 70.6%) at 5 years from HCC diagnosis; it was less frequent in males, p=0.036, while all the other variables were similarly distributed between PLWH with RE or no-RE.
Overall, 2-and 5-year survival probabilities after HCC diagnosis were 72% (55.1% – 83.4%) and 48% (31.7% – 62.7%) respectively. A higher rate of survival was observed in participants receiving OLT: 2- and 5-year survival was 100% and 90.9%, respectively, in comparison to other therapies (LR, RFTA, CRE) (60.9% and 30.6%, respectively) log-rank p=0.0006. The 2-and 5-year survival in participants with no-RE was 70.5% (45.7% – 85.6%) and 54.6% (IQR 30.6% -73.4%) respectively, and 73.7% (IQR 47.9 – 88.1) and 42.1% (20.4% – 62.5%) among RE, respectively, log-rank p=0.7772.
By multivariate analysis AFP at values < 28.8 ng/mL, at HCC diagnosis, was the only factor predicting survival.

Conclusion:
Fifty percent of PLWH were alive at five years from HCC diagnosis with 90.9% among OLT patients. Recurrence after IT was observed in 46% of HCC/PLWH. Overall survival did not seem to be affected by HCC recurrence. AFP cut off levels 28.8 ng/mL at HCC diagnosis was the only independent variable associated with survival.

Figure:

Table 1. Characteristics at HCC diagnosis of PLWH receiving invasive therapy according to the primary study outcome (died or alive)

Variable		Overall (number=41)	Died (number=22)	Alive (number=19)	P value
Age, years		53 (49 - 56)	53 (49 - 56)	54 (50 - 58)	0.267
Sex, male		34 (83)	16 (72)	18 (95)	0.010
Years since first ART		11.95 (7.29 - 16.84)	10.64 (5.11 - 15.9)	14.95 (8.78 - 19.39)	0.206
Number of nodules>3		3 (7)	3 (14)	0 (0)	0.010
BCLC	0/A	20 (48.8)	7 (31.8)	13 (68.4)	0.063
	B	8 (19.5)	6 (27.3)	2 (10.5)	
	C/D	13 (31.7)	9 (40.9)	4 (21.1)	
Treatment					0.0038
	OLT	11 (26.8)	1 (4.5)	10 (52.6)	
	LR	6 (14.6)	4 (18.2)	2 (10.5)	
	RFTA	6 (14.6)	3 (13.6)	3 (15.8)	
	CRE	18 (43.9)	14 (63.6)	4 (21.0)	
AFP, ng/mL		27.8 (8.7 - 135.2)	41.4 (14.6 - 347.7)	14.4 (7.3 - 23.95)	0.015
AFP, ng/mL	<28.8	19 (51.4)	6 (28.6)	13 (81.3)	0.002
	≥28.8	18 (48.6)	15 (71.4)	3 (18.8)	
AST, U/L		70 (34 - 100)	71 (38 - 100)	45 (31 - 158)	0.855
ALT, U/L		67 (33 - 99)	64 (32 - 99)	68 (35 - 104)	0.466
Bilirubin, mg/dL		1 (0.78 - 1.75)	1 (0.78 - 1.52)	1 (0.78 - 1.75)	0.824
CD4 cells count, mmc		433 (262 - 722)	333.5 (246 - 722)	530 (345 - 764)	0.218
CD8 cells count, mmc		732 (445 - 1209)	589 (307 - 757)	1021 (621 - 1392)	0.02
Platelets count, 10^9/L		107 (64 - 141)	100 (64 - 123)	110.5 (63 - 154)	0.325
PTs/INR		1.06 (1.03 - 1.28)	1.06 (0.99 - 1.37)	1.08 (1.05 - 1.21)	0.953
Creatinine, mg/dL		0.74 (0.68 - 0.86)	0.72 (0.66 - 0.88)	0.75 (0.72 - 0.86)	0.444

Results are described by median (IQR) or frequency (%). Abbreviations: ART: antiretroviral therapy; AFP: alpha phetoprotein; PTs/INR: prothrombin in second / international normalized ratio; AST: aspartate aminotransferase (normal values<35 U/L); ALT: alanine aminotransferase (normal values<59 U/L); BCLC: Barcelona Clinic Liver Cancer.

P07-13
It takes a team for HCC: improvement of outcome with the multidisciplinary ambulatory for systemic therapy

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Background and Aims: Hepatocellular carcinoma (HCC) is the major cause of liver-related death worldwide. In the last years, a new approach with tyrosine kinase inhibitors (TKIs) and immune checkpoint inhibitors (ICIs) started to gain attention in HCC setting. With these new therapies multidisciplinary team (MDT) discussion becomes necessary due to increased curative treatments, frequency of stage migration, higher treatment rates and reduced mortality. In Verona Hospital, in addition to MDT discussion, a LIVER-MDT ambulatory was created, to our knowledge the first in the Italian health system, with an oncologist, an hepatologist, a surgeon, and an internist. Aim of this study was to verify if the LIVER-MDT ambulatory is useful to reduce adverse effects and mortality compared to a traditional ambulatory (ELEVETOR cohort, Liver cancer 2022).

Method: we collected data from patients attending the LIVER-MDT ambulatory. Major and minor adverse effects (MAE and mAE) and antitumoral doses were collected. Death and progression free survival (PFS) were also recorded.

Results: Among 834 patients evaluated at the MDT discussion from 2021, 40 patients were referred to the LIVER-MDT ambulatory to start systemic treatment. Median age was 69.5 (53-82), 82.5% were male. Cirrhosis etiology was 45% viral (HCV/HBV), 37.5% MAFLD and 10% alcohol. Compared to ELEVATOR cohort, less MAE were recorded (20% vs 32.7%, $p<0.01$). In addition, MAE strictly due to the systemic therapy developed in 7.5%. No patients developed uncontrolled and resistant arterial hypertension or heart failure during the treatment. 32.5% died, with a median PFS of 8.85±6.03 (median in ELEVATOR 6.4). Only 15 % of patients needed a reduction of antitumoral drug dose, compared to 50% in ELEVATOR

Conclusion: LIVER-MDT ambulatory improves the outcome of HCC patients on systemic therapy, reducing MAEs and mAEs, in particular cardiovascular complications, and seems the best approach for the increasing number of HCC patients.

P07-15
NMS-01940153E, an MPS1 inhibitor with anti-tumor activity in relapsed or refractory unresectable hepatocellular carcinoma

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Background and Aims: Monopolar Spindle 1 (MPS1) kinase guides proper division of chromosomes during mitosis. It is overexpressed in several tumors, including hepatocellular carcinoma (HCC), where it correlates with negative clinical and tumor features. NMS-01940153E (NMS-153) is a highly potent and selective inhibitor of MPS1 kinase with long residence time and strong preclinical antitumor activity in different tumor types. It was tested in a FIH study (EudraCT 2014-002023-10), where activity in HCC was detected. The Sponsor decided to further explore preclinical and clinical activity in HCC

Method: A panel of 6 HCC cell lines was assessed for antiproliferative activity in a 2D colony forming assay.MPSA-153-001 is a Phase 1/2 trial (EudraCT 2020-001002-26) with NMS-153 administered as single agent intravenously, on days 1,8,15 in a 4 wk-cycle, in patients with unresectable HCC previously treated with systemic therapy. In the phase 1, a 3+3 escalation design started at 100 mg/m2/wk, to determine MTD and RP2D

Results: In HCC lines, NMS-153 showed ~2-Log higher antiproliferative activity compared to sorafenib, lenvatinib, and regorafenib. We present results from the phase 1 part of the trial (data cut-off date, 02-Dec-2022). Twelve HCC patients were enrolled. Median age was 64 years, median number of prior systemic therapies was 2 (range 1-3). Two DLTs (G4 neutropenia with either G4 sepsis or G2 urinary tract infection) occurred at 135 mg/m2/wk. Most frequent (≥10%) any grade drug-related treatment emergent adverse events (TRAEs) were neutropenia (50% in the overall population, 2/6 patients at 100 mg/m2/wk, all G≥3), chromaturia and thrombocytopenia (25%), anemia, asthenia, diarrhea, and injection site reaction (16.7%). Neutropenia was rapidly reversible from onset of ≥G3 to ≤G1 in 9.5 days average.

Of 11 patients evaluable for efficacy, two (one for each dose level) had confirmed investigator-assessed PRs with duration of 2.5 and 9.3 months; both discontinued treatment due to PD at 6.5 and 11.1 months from treatment start, respectively. Two further patients, one for each dose level, had durable SDs, one progressing after 10.9 months from treatment initiation, and one still on treatment 20.8 months after enrollment. Three patients, two with PR and one with SD, showed AFP decrease.

The PK profiles of parent and metabolite showed an increase in exposure with the dose with approximately 4-day half-life for the parent drug; PK at the RP2D was in a meaningful, active range relative to preclinical predictions.

Conclusion: NMS-153 targets a novel mitotic mechanism and showed preclinical and clinical activity in HCC, including two confirmed PRs. Safety features were reasonable, with manageable and reversible neutropenia as the most frequent any grade TRAE. NMS-153 is currently under evaluation in patients with unresectable HCC previously treated with at least 1 immunotherapy as first line and 1 TKI line

P07-17
Rising admissions and treatments for hepatocellular carcinoma in Irish hospitals (2009-2020)

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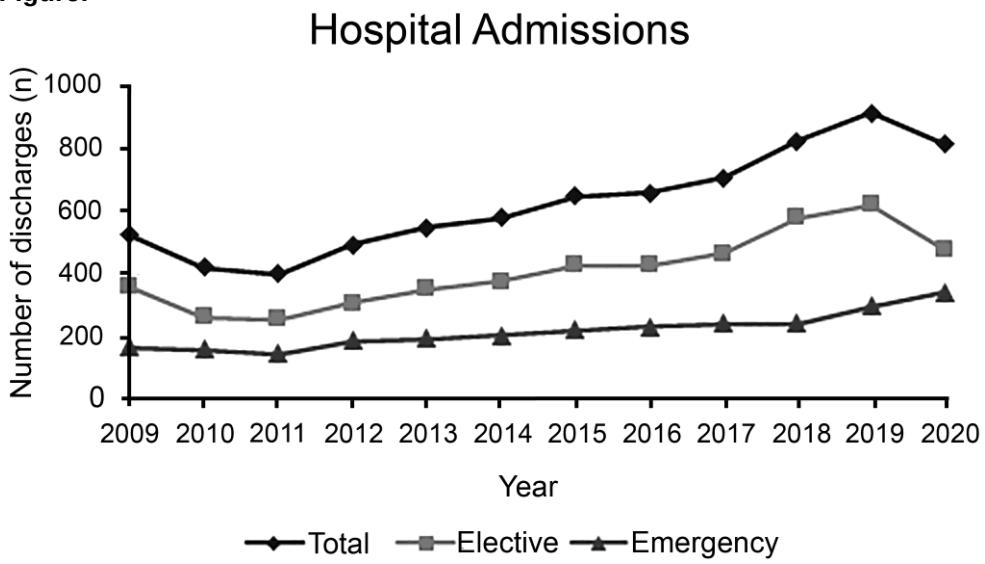
Background and Aims: The incidence of hepatocellular carcinoma (HCC) is increasing. The health service-related burden stemming from this in Ireland is unclear. The aim of this study was to quantify admissions to public hospitals in Ireland with a diagnosis of HCC, and identify associated diagnoses, treatments and outcomes.

Method: The national health information system, Hospital In-Patient Enquiry (HIPE) scheme, used to collect data on discharges and deaths from all acute public hospitals in Ireland was interrogated. For this study, HIPE records with ICD-10-AM code *C22.0 Liver cell carcinoma (Hepatocellular carcinoma/ Hepatoma)* listed as a primary or additional diagnosis from 2009 – 2020 were included. Uncoded and maternity cases were excluded.

Results: There was a total of 7,507 discharges over the 12-year period. Admissions increased from 399 in 2011 to 912 in 2019. 2020 saw a COVID related decrease. 35% of admissions were emergencies. 80% were male. Mean age was 64.8 years. Excluding day-cases, the average length of stay was 9.8 days. The mortality rate for elective admissions reduced from 2.81% in 2009 to 0.81% in 2019. The mortality rate for emergency admissions remained unchanged, 15.76% in 2009 and 14.63% in 2019. HCC treatments included transarterial chemoembolization (TACE) (n=1253), intravenous chemotherapy (n=877), surgical resection (n=187), liver transplant (n=152), percutaneous ablation (n=123) and radio-embolization (n=32). Transplant numbers were stable while TACE, resection, and percutaneous ablation all increased until 2020.

Conclusion: HCC places a high and increasing burden on Irish inpatient services. HCC related admissions doubled over the study period, mostly from an increase in elective treatments. These patients remain in hospital almost twice the national average length of stay. Elective-admission related mortality has fallen while emergent mortality is static.

Figure:


Hospital Admissions
Number of discharges (n)
1000
800
600
400
200
0
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
Year
Total
Elective
Emergency

P07-19
Varices and thrombocytopenia are predictors of survival outcome after liver resection for hepatocellular carcinoma (HCC): a twenty-year retrospective study

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Background and Aims: Surgical resection is a curative treatment for early hepatocellular carcinoma (HCC). Portal hypertension and varices are established predictors of poor prognosis in HCC resection. Recently thrombocytopenia has been proposed as a prognostic marker. Our study is the first UK retrospective analysis aiming to identify prognostic factors for HCC resection, with a particular focus on varices and thrombocytopenia.

Method: Patients undergoing curative HCC resection at a single UK tertiary HCC centre between 2002 and 2022 were included. Patient characteristics including demographics, varices, thrombocytopenia (platelet count<100 x10^9/L), lesion number, Barcelona Clinic Liver Cancer Score (BCLC) and histological features including tumour grade, vascular invasion and resection margin were reviewed. Overall survival (OS) and disease-free survival (DFS) were calculated. Additionally, we performed univariate and multivariate Cox regression on baseline patient characteristics and histological features.

Results: 108 patients underwent resection within the study period. The median cohort OS and DFS was 93.9 months and 24.7 months respectively. Median OS was reduced in both thrombocytopenia (42.0 vs 95.1 months, p = 0.04) and in the presence of varices (22.5 vs 95.1 months, p = 0.01). Median DFS was lower in thrombocytopenia (14.3 vs 28.1 months, p = 0.13) and varices (median DFS 5.8 vs 27.3 months, p = 0.02). In univariate analysis, thrombocytopenia, varices, increasing age, intermediate BCLC stage, lower serum albumin, increasing MELD-Na and multiple lesions were significant predictors for reduced OS. In multivariate analysis, varices were associated with reduced OS (OR 7.42, 95% CI 1.62-34.0, p = 0.01) and reduced DFS (3.43, 95% CI 1.04-11.29, p = 0.04). Poor tumour grading predicted reduced overall survival in multivariate analysis (OR 8.68, 95% CI 1.43-52.78, p = 0.02).

Conclusion: The presence of pre-treatment thrombocytopenia, and varices are associated with reduced OS in HCC patients undergoing curative-intent resection Our study highlights the importance of thrombocytopenia and varices in prognostication models and advocates variceal screening prior to surgery.

Figure:

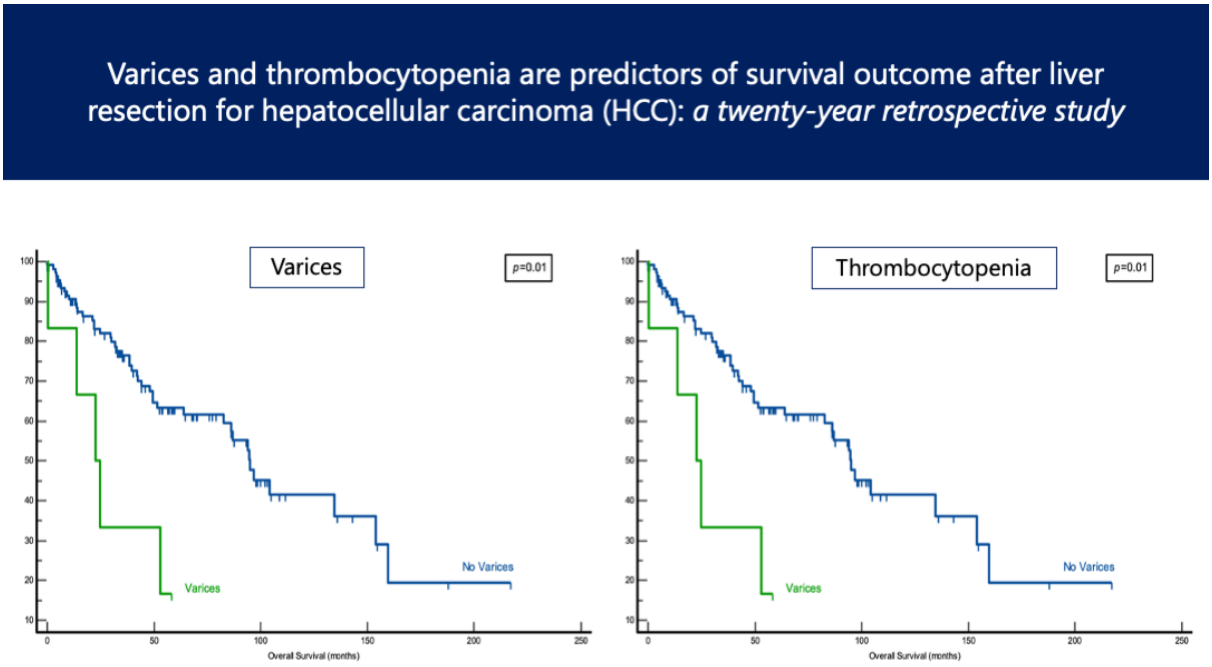

Figure 1 showing significantly reduced overall survival in patients with baseline varices and thrombocytopenia prior to hepatic resection

P07-20
Progression free survival of first-line systemic therapies for advanced hepatocellular carcinoma: A network meta-analysis

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Background and Aims: This research aimed to compare the first-line systemic therapies for advanced hepatocellular carcinoma (HCC) in terms of progression-free survival (PFS).

Method: Embase, MEDLINE, and CENTRAL were searched to identify randomized controlled trials assessing first-line systemic therapies for treating adults with locally advanced or metastatic unresectable HCC. Clinical and statistical heterogeneity assessment was performed using trial, patient, and outcome level covariates. Network meta-analysis (NMA) used generalized linear models with random effects within a Bayesian framework using informative priors. The risk of bias was performed using the funnel plot and Egger's regression test. The proportional hazards assumption was also tested for the appropriateness of conventional HR-based NMA.

Results: A total of 12 RCTs assessing first-line chemotherapy, molecular targeted therapy, or immunotherapy, as mono or combination therapies, were included in the NMA. Most studies were conducted in a Global (n=8) setting, followed by Asian (n=3) and non-Asian (n=1) settings. Compared with placebo, sintilimab + bevacizumab [HR, 95% CI: 0.32, 0.17-0.63], camrelizumab + rivoceranib [0.3, 0.16-0.57], atezolizumab + bevacizumab [0.37, 0.19-0.7], tremelimumab + durvalumab [0.52, 0.27-0.99], lenvatinib + pembrolizumab [0.32, 0.14-0.71], cabozantinib + atezolizumab [0.37, 0.18-0.73], sorafenib [0.58, 0.39-0.85], lenvatinib [0.38, 0.2-0.73], and nivolumab [0.54, 0.28-0.998], were associated with significantly better PFS. Compared with sorafenib, most treatments showed numerically better but statistically non-significant PFS. SUCRA rankings were generally better for combination therapies followed by monotherapies and placebo.

Conclusion: The promising results of immunotherapy combinations with TKIs and other agents indicate the availability of more first-line options in the near future for advanced HCC patients.

P08-01
Lower risks of sodium glucose cotransporter 2 (SGLT2) inhibitors compared to dipeptidyl peptidase-4 (DPP4) inhibitors for hepatocellular carcinoma in type 2 diabetes mellitus: A population-based study

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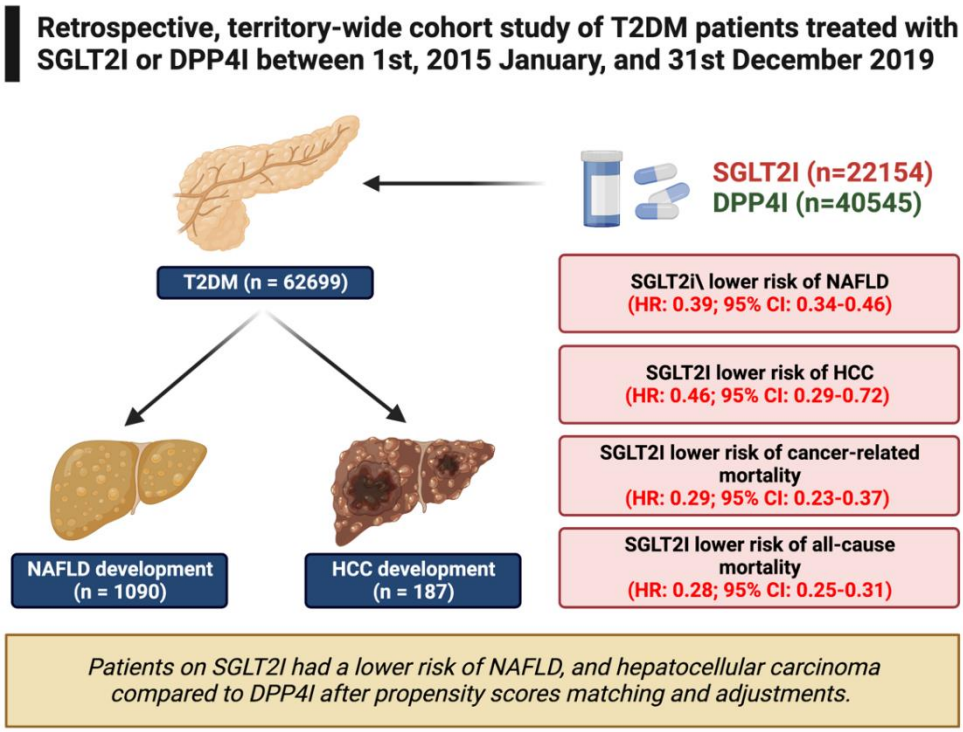
Background and Aims: Diabetes mellitus (T2DM) is one of the major global public health concerns. It was suggested to be a risk factor of hepatocellular carcinoma (HCC). The association between sodium glucose cotransporter 2 inhibitors (SGLT2I) versus dipeptidyl peptidase-4 inhibitors (DPP4I) and the risks of hepatocellular carcinoma (HCC) are currently unknown. The aim of this study is to compare the differences in new-onset acute HCC between SGLT2I and DPP4I users in T2DM.

Method: This was a retrospective population-based cohort study including type-2 diabetes mellitus (T2DM) patients treated with either SGLT2I or DPP4I between 1st January 2015 and 31st December 2019 in Hong Kong. Patients with concurrent DPP4I and SGLT2I usage were excluded. The primary outcome was HCC. Propensity score matching (1:1 ratio) was performed using the nearest neighbour search. Univariable and multivariable Cox regression was applied to identify significant predictors. Competing risks models and multiple approaches using the propensity score were performed.

Results: This cohort included 62699 T2DM patients, amongst which 22154 patients were on SGLT2I and 40545 patients were on DPP4I. After matching (44308 patients), 187 patients developed HCC (Incidence: 0.8; 95%CI: 0.7-0.9). Overall, SGLT2I was associated with lower risks of HCC (HR: 0.46;95%CI: 0.29-0.72) compared to DPP4I after adjustments. SGLT2I was also associated with lower risks of cancer-related mortality (HR: 0.29;95%CI: 0.23-0.37) and all-cause mortality (HR: 0.28;95%CI: 0.25-0.31). However, amongst patients with hepatitis B virus infection, SGLT2I was associated with higher risks of HCC (HR: 3.28;95%CI: 1.21-8.90). The results were consistent in different risk models and matching approaches.

Conclusion: SGLT2I was associated with lower risks of HCC compared to DPP4I after adjustments.

Figure:


Retrospective, territory-wide cohort study of T2DM patients treated with SGLT2I or DPP4I between 1st, 2015 January, and 31st December 2019
SGLT2I (n=22154)
DPP4I (n=40545)
T2DM (n = 62699)
SGLT2i\ lower risk of NAFLD
(HR: 0.39; 95% CI: 0.34-0.46)
SGLT2I lower risk of HCC
(HR: 0.46; 95% CI: 0.29-0.72)
SGLT2I lower risk of cancer-related mortality
(HR: 0.29; 95% CI: 0.23-0.37)
SGLT2I lower risk of all-cause mortality
(HR: 0.28; 95% CI: 0.25-0.31)
NAFLD development
(n = 1090)
HCC development
(n = 187)
Patients on SGLT2I had a lower risk of NAFLD, and hepatocellular carcinoma compared to DPP4I after propensity scores matching and adjustments.

P08-02-YI
Transarterial chemoembolization and systemic treatment in patients with autoimmune liver disease-associated hepatocellular carcinoma: Outcome and safety profile

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Background and Aims: Hepatocellular carcinoma (HCC) develops in patients with autoimmune liver disease (AILD) such as autoimmune hepatitis (AIH) and primary biliary cholangitis (PBC). Due to the low incidence of AILD, this subgroup is regularly underrepresented in HCC clinical trials. Data on treatment tolerability and prognosis in these rare liver patients is scarce. Hence, the aim of this study was to investigate, whether patients with HCC-AILD will equally benefit from systemic treatment with tyrosinkinase inhibitors (TKIs) or transarterial chemoembolization (TACE) and demonstrate a similar safety profile compared to patients with HCC due to viral, or non-/alcoholic liver disease.

Method: For this European retrospective study, conducted by the ERN Rare Liver, we initially enrolled 107 patients with HCC-AILD (55 x AIH, 52 x PBC) treated at 13 centers from 1996 to 2020. Of these, 72 remained for the final analysis (exclusions criteria: treatment other than TACE or TKIs, 38 x AIH, 34 x PBC). Propensity score matching 1:1 with a pool of 347 non-AILD associated HCC patients from Hamburg was conducted to adjust for differences in major clinical confounders between the two groups. Subsequently, comparative analyses of median overall survival (mOS) and treatment tolerability were performed, thereby applying a sequential analysis method for patients having undergone both TACE and systemic treatment.

Results: The final propensity-matched cohort included a total of 130 patients who were treated with TACE and 56 with systemic treatment. HCC-AILD patients demonstrated a comparable mOS for both TACE (19.5 months [10.1 – 28.3] vs 22.1 months [11.4 – 30.2], p = 0.9) and systemic treatment with TKIs (15.4 months [5.3 – na] vs 15.1 months [9.4 – 35], p = 0.5). For TACE, adverse events (AE) occurred less frequently in HCC-AILD patients than in controls (e.g. post-TACE embolization syndrome) (≥ 1 AE: 34 % vs 62 %, p = 0.003), whereas there was no significant change in rate of AEs for systemic treatment (≥ 1 AE: 68 % vs 82 %, p = 0.2).

Conclusion: In conclusion, we present the first study, investigating the outcome and safety profile of rare liver patients with HCC-AILD treated with TACE or TKIs. Patients with HCC-AILD have similar mOS to both local and systemic treatment, and a more favorable tolerability compared to non-AILD associated HCC. Due to the exclusion of HCC-AILD patients in recent immunotherapy trials, systemic treatment with TKIs will continue to be the standard of care for HCC-AILD.

P08-06
A prognostic nomogram of CDKs/CDKLs-related genes for hepatocellular carcinoma

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Background and Aims: Hepatocellular carcinoma (HCC) is the most common malignant tumor of the liver. Anti- cyclin-dependent kinases (CDKs) therapy has shown effectiveness in several kind of cancers, however, The effect of CDKs and cyclin-dependent kinases like (CDKLs)-related genes on the clinical prognosis of HCC is still unclear. In this study, we aims to build a prognostic nomogram of CDKs/CDKLs-related genes for hepatocellular carcinoma.

Method: The CDKs/CDKLs genes were obtained from PUBMED articles and the molecular signatures database. The prognosis nomogram of six related genes were constructed using the last absolute shrinkage and selection operator (LASSO), and Receiver Operating Characteristic (ROC) curves were used to evaluate the signature. All these analyses were validated using multiple datasets of the International Cancer Genome Consortium (ICGC).

Results: The CDKs/CDKLs genes (CDK1, CDK4, CDK5, CDK16, CDKL3, CDKL4) were finally included. According to the risk score, the patients were divided into high-risk and low-risk groups. Survival analysis showed that the overall survival (OS) of the high-risk group was significantly lower than that of the low-risk group. Based on the risk score and the clinical features of age and clinical stage, we established a risk prognostic nomogram to predict the OS of patients with HCC. The ROC curves and Calibration curves in the training set and validation set showed that this nomogram had a good degree of discrimination and calibration.

Conclusion: In this study, we established a new prediction signature of six genes related to CDKs/CDKLs genes, which can effectively predict the prognosis of HCC patients.

Figure:

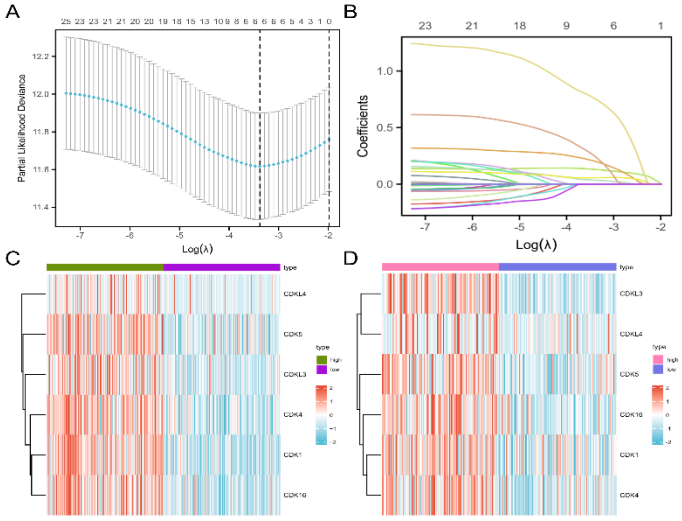

Figure 1. (A).LASSO coefficient profiles of the 26 CDKs/CDKLs genes from the TCGA cohort. (B) Partial-likelihood deviance of variables revealed by the LASSO regression signature. Red dots represent the partial likelihood of deviance values, gray lines represent the standard error (SE), and the two vertical dotted lines on the left and right represent optimal values by minimum criteria and 1 – SE criteria, respectively. (C) TCGA heatmap of the prognostic signature consisting of six CDKs/CDKLs genes. (D) ICGC heatmap of the prognostic signature consisting of six CDKs/CDKLs genes.

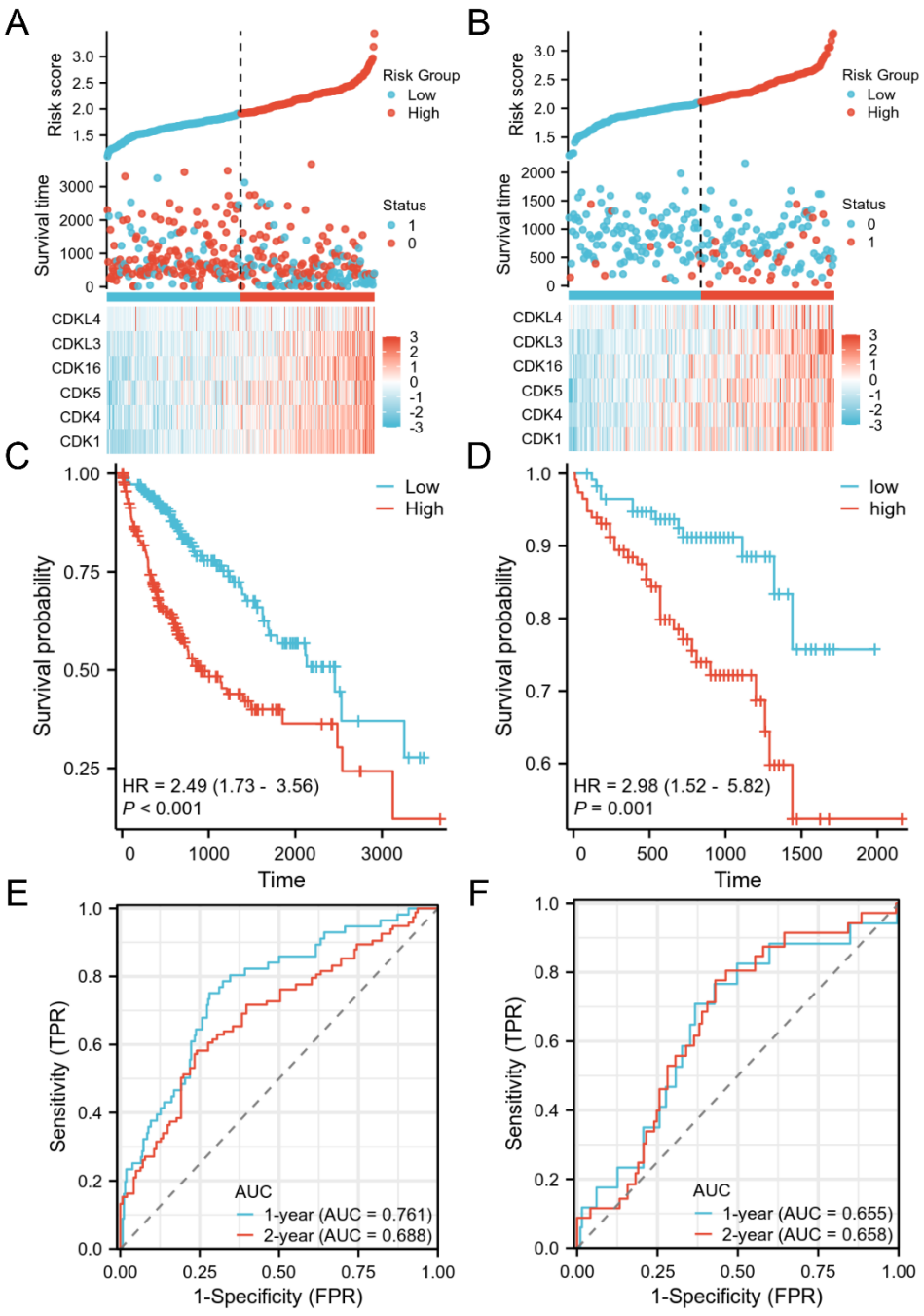

Figure 2. Prognostic analysis of the 6-gene signature in the TCGA and ICGC cohort. (A,B) Distribution of ri score distribution of overall survival in high-risk and low-risk groups the TCGA (A) and ICGC (B) cohort; (C,D) Kaplan – Meier curve of the 6-gene signature in the TCGA cohort (C) and ICGC cohort (D) (P<0.05). (E,F) ROC analysis for risk signature at 1, 2 year survival time in the TCGA cohort (E) and ICGC cohort (F)

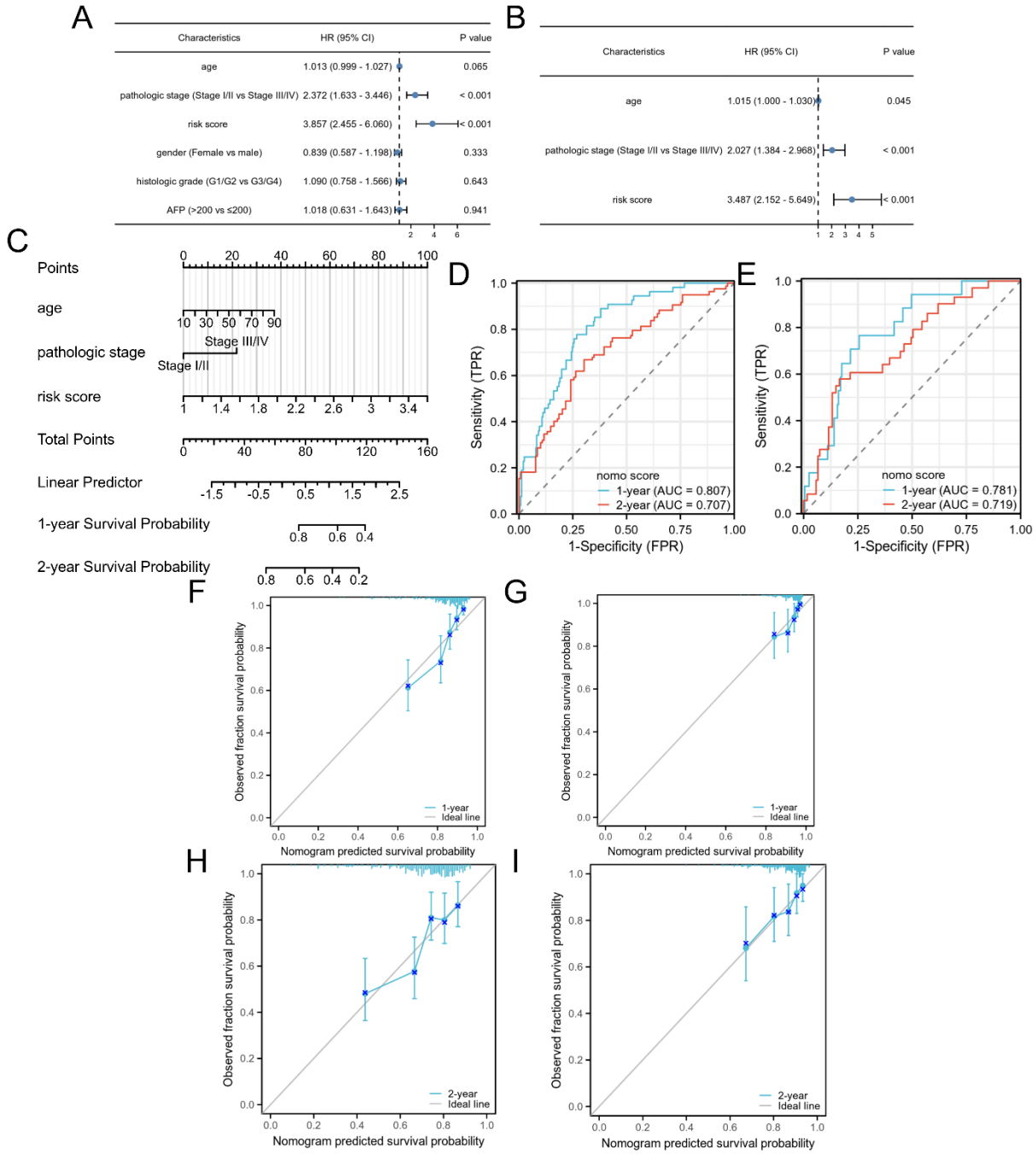

Figure 3. Univariate Cox regression analyses (A) of overall survival and multivariate Cox regression analyses (B) of overall survival in the TCGA cohorts. (C) Nomograms for predicting 1,2-year survival in the TCGA cohort. (D,E) ROC curves for 1, 2-year time showing the comparison between the survival prediction ability of nomogram D: TCGA, B: ICGC.(F,H) Calibration curves for the nomograms the TCGA cohort. (G,I) Calibration curves for the nomograms the ICGC cohort.

P08-07
Baveno VI and VII criteria are not suitable for screening of large size esophageal varices and clinically significant portal hypertension in patients with HCC

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Background and Aims: Baveno VI and VII criteria are used in patients with cirrhosis to rule-out large size varices (EV) and rule-in/out CSPH. Their diagnostic performance is still unclear in patients with HCC.

Method: All Child-Pugh A cirrhotic patients with HCC with endoscopy, liver stiffness measurement (LSM) and platelet count within 6 months were retrospectively included and classified according to the BCLC stage in 2 centers. Favorable Baveno VII criteria were defined by LSM < 20kPa and Plt > 150G/l, favorable Baveno VII criteria if LSM ≤ 15kPa and Plt ≥ 150G/l. Clinical significant portal hypertension (CSPH) was defined by a HVPG ≥ 10 mmHg or the presence of EV regardless the size.

Results: 185 Child-Pugh A cirrhotic patients were included in the study (male 87%, median age 63 years, etiology of cirrhosis alcohol/metabolic syndrome/hepatitis C/hepatitis B in 46%/36%/20%/31% of cases and mixed for 33% of patients). Esophageal varices (EV) were present in 44% of patients (23% large size EV) and HVPG≥10 mmHg in 41.7% (mean HVPG 8 mmHg). Median platelet count and elastometry were 148x103/mm3 and 25 kPa respectively, 50% had platelet count <150x103/mm3.
In the cohort, 46% of patients were classified as BCLC-0/A, 28% as BCLC-B and 26% as BLCL-C, and 18% had received prior treatment for HCC. A multinodular and infiltrative form was present in 52% and 15% of patients respectively, with a location in the right liver in 78% of cases. Compared to BCLC O-A HCC (21kPa), elastometry was higher in BCLC-B (25kPa, p=0.005) and BCLC-C (27kPa, p<0.001) patients. There was no difference in platelet count between the different groups.
In patients with favorable Baveno VI criteria, 7.8% in the whole cohort (Se 93%, PNV 82%), 11.1% of BCLC-0-A (Se 89%, PNV 89%) and 10.0% of BCLC-C patients (Se 91%, PNV 90%) had large EV.
Among the patients with HVPG<10 mmHg, i.e. without CSPH, 5.7% had large size EV and 17.1% small EV.
CSPH was present in 26.7% of patients with favorable Baveno VII criteria in the whole cohort and in 23.5% of those of the BCLC-0/A subgroup.
Specificity of LSM≥25kPa to rule-in CSPH was of 63%.

Conclusion: Favorable Baveno VI criteria are not appropriate to rule-out the presence of high-risk EV and Baveno VII criteria to rule-in/out CSPH in HCC patients

P08-08
Impact of sarcopenia on survival of patients with hepatocellular carcinoma treated with sorafenib

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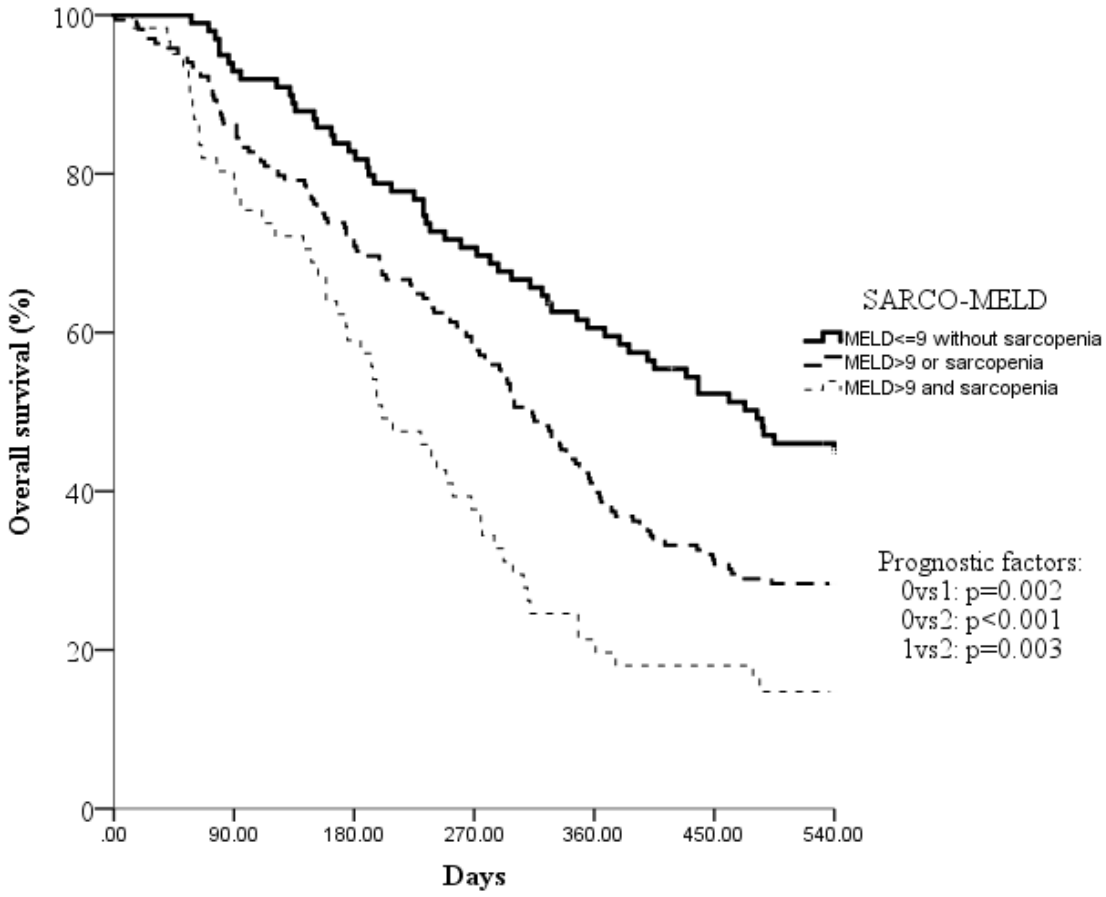
Background and Aims: Some studies have demonstrated that sarcopenia, defined as a loss of skeletal muscle mass and function, is frequent and represents a prognostic factor in patients with hepatocellular carcinoma (HCC). There are controversial data about the prognostic significance of sarcopenia in patients with HCC treated with sorafenib, and most of the studies come from Asian series. Therefore, the aims of the present study were: 1) to investigate whether sarcopenia has an independent prognostic meaning in European patients with HCC undergoing sorafenib therapy; 2) to define a predictive model of the death risk in this setting.

Methods: A training set of 215 and a validation set of 113 cirrhotic patients with HCC undergoing sorafenib and with abdominal Computed Tomography (CT) scans performed within 8 weeks from treatment start were retrospectively analysed.
Sarcopenia was defined as a reduction in the skeletal muscle index (SMI) assessed by a CT scan performed before sorafenib start. SMI was calculated on a CT transverse image crossing the 3rd lumbar vertebra.
Male patients with SMI <53 cm2/m2 with a body mass index (BMI) ≥25 or with SMI <43 cm2/m2 with a BMI <25, and female patients with SMI <41 cm2/m2 regardless of BMI were identified as having sarcopenia. The primary outcome was overall survival (OS) at 540 days. The Kaplan-Meier method was used to estimate survival curves and they were compared using the log-rank test. Univariate and multivariate analyses for OS were made using the Cox proportional hazards model and hazard ratio (HR) and corresponding 95% confidence interval (95%CI).

Results: Sarcopenia was present in 103 patients (47.9%) in the training set and in 56 patients (49.6%) in the validation set.
The two groups were not entirely matched, as differences were found in BMI, INR value, serum albumin, Model for End-Stage Liver Disease (MELD) score, alpha-fetoprotein (AFP) levels, and performance status (PS). No differences were found in tumor burden except for a more frequent macrovascular invasion (MVI) in the training group (33.5% vs 18.5%, p=0.005).
The Cox analysis selected as independent prognostic factors significantly associated with survival MELD score >9 (HR 1.46, 95%CI 1.07-1.99, p= 0.016 and HR 1.09, 95%CI 1.02-3.53, p=0.044; respectively) and sarcopenia (HR 1.46, 95%CI 1.05-2.02, p=0.024 and HR 2.07, 95%CI 1.26-3.43, p=0.004; respectively), both in training and validation set.
Therefore, we assembled a prognostic indicator named “SARCO-MELD” based on the two independent aforementioned factors creating 3 groups with a different risk of death: group 1 (0 prognostic factors), group 2 (1 factor) and group 3 (2 factors).

Conclusion: This study demonstrated that sarcopenia is an independent prognostic factor in patients with HCC undergoing sorafenib therapy, and its association with a MELD score >9 identifies patients with a very poor outcome despite the treatment.



Patients at risk

Prognostic factors (n)							
0:	99	92	82	70	59	50	44
1:	168	145	121	98	68	52	47
2:	61	49	36	23	13	11	9

P08-12
Temporal trends in Hepatocellular carcinoma in Pakistan

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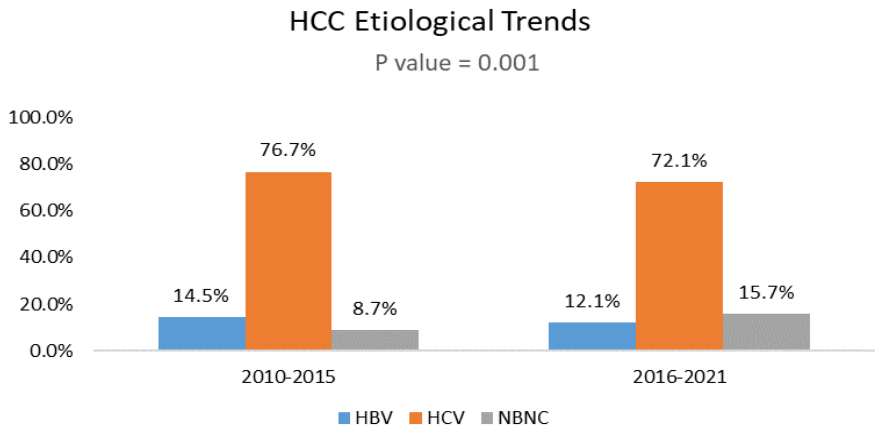
Background and Aims: The incidence of hepatocellular carcinoma (HCC) has been spiraling upwards making it the fifth most common malignancy and third most leading cause of cancer-related mortality throughout the world. Recently, a shift in etiology of HCC has been noted towards non-viral causes hence we aimed to identify this change in our region.

Method: A cross-sectional study was done at Aga Khan Hospital, Karachi, Pakistan.

Results: A total of 2312 HCC patients were divided in Group 1 (2010-2015) and Group 2 (2016-2021) containing 1174 and 1138 patients respectively. In group 1, the mean age was 57.5 +/- 10.71 years while the mean age in group 2 was 59.3 +/- 10.4 years. Group 1 consisted of 790 males (67.29%) while females were 384 (32.79%). The number of males in group 2 was 788 (69.24%) and there were 350 females (30.75%). The etiological trends in group 1 showed 901 (76.74%) HCV-Chronic liver disease (CLD) cases, 170 (14.48%) HBV-CLD cases and 103 (8.7%) cases due to non-B Non-C CLD. Group 2 revealed 821 (72.14%) HCV-CLD cases, 138 (12.12%) HBV-CLD while that of Non-B Non-C CLD cases were 179 (15.72%), almost twice that found in group 1 with statistically significant p-value of <0.001.

Conclusion: This is an important study reflecting a significant hike in cases of Non-B Non-C CLD-related HCC in Pakistan over the past couple of years, which is in accordance with the global data.

Figure:



P08-15
Tolerance and efficacy of the early switch towards atezolizumab + bevacizumab after failure of selective internal radiation therapy for hepatocellular carcinoma

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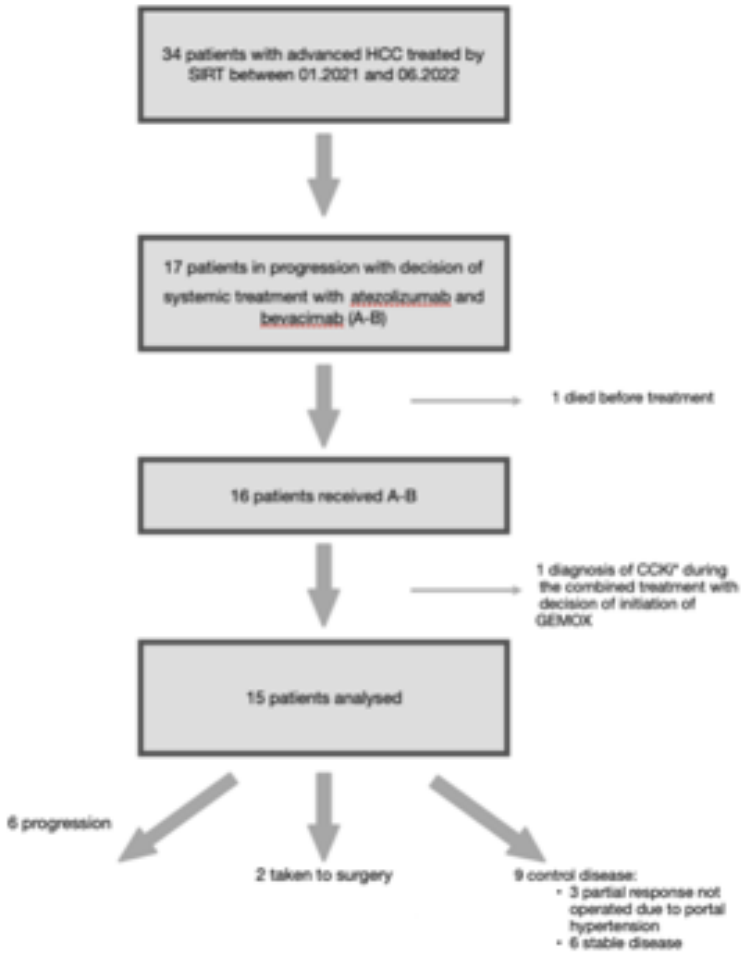
Background and Aims: The selective internal radiation therapy (SIRT) is an option of treatment for intermediate or for some advanced hepatocellular carcinoma (HCC) without extrahepatic metastasis. Atezolizumab - bevacizumab (A-B) has been approved as the reference treatment of advanced HCC worldwide. Morever, the latter might also modulate immunological effects of SIRT. We assessed the tolerance and efficacy of the early switch towards A-B systemic treatment in a series of patients with non resectable HCC after failure of SIRT.

Method: Sixteen consecutive patients with intermediate or advanced HCC were followed in the at Paul Brousse hospital between january 2021 and june 2022. They received atezolizumab (1200 mg intravenous IV) + bevacizumab (15 mg/kg IV) every 3 weeks after failure of a SIRT defined by either an increase of AFP with the persistence of an active hyper-vascular lesion at least 3 months after SIRT or progression of the treated lesion(s) or new HCC lesions regardless of the time after SIRT. We analysed the tolerance and the disease control rate (DCR) under A-B.

Results: The main clinical features were as follows : 88% male, median age 76.5 years, Performans statuts < 2, Child-Pugh score < B7, 52% BCLC-C, 64% tumor progression out of the SIRT areas, 0% extrahepatic metastasis. There was no new signal concerning the safety. Median time between SIRT and A-B was 119 days (95%CI 58-194). The median follow-up was 11 months (95% CI 7-15) and median duration of A-B treatment was 7 months (95% CI 3-13). The control disease rate was 56% (9/16) (partial response PR 19 %, stable disease SD 37%) during a median duration of 10 months (95% CI 7-13). Two patients with SD had partial hepatectomy after 6 months under A-B but all those with PR had significant portal hypertension that contra-indicated curative surgery.

Conclusion: Early A-B systemic treatment as a rescue after SIRT failure for intermediate or advanced HCC is well tolerated and may offer a re-stabilization of HCC and even curative surgery in a few.

Figure:


34 patients with advanced HCC treated by SIRT between 01.2021 and 06.2022
17 patients in progression with decision of systemic treatment with atezolizumab and bevacizumab (A-B)
1 died before treatment
16 patients received A-B
1 diagnosis of CCK/" during the combined treatment with decision of initiation of GEMOX
15 patients analysed
6 progression
2 taken to surgery
9 control disease:
• 3 partial response not operated due to portal hypertension
• 6 stable disease

P08-16
INTERLEUKIN-6: a new marker of advanced-sarcopenic HCC cirrhotic patients

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Background and Aims: Hepatocellular carcinoma (HCC) is a major cause of liver cancer-related death worldwide. It usually occurs in cirrhosis with different etiopathogeneses. Sarcopenia is present in almost one third of patients with HCC and is a strong and independent risk factor for mortality. Interleukin-6 (IL-6) is a proinflammatory cytokine that increases considerably in pathological settings such as trauma, inflammation and neoplasia. Based on pre-clinical data in HCC, IL-6 signaling leads to tumor progression or local recurrence. Our aim was to investigate whether IL-6 is correlated with HCC stage and could represent a diagnostic marker for sarcopenia.

Method: 93 HCC cirrhotic patients in different stages according to BCLC-2022 (stages A-B-C), were enrolled. Anthropometric and biochemical parameters, comprehensive of IL-6, were collected. Skeletal Muscle Index (SMI) was measured by a dedicated software on computer tomography (CT) images. Sarcopenia cut-off values derived from cirrhotic patients on the liver transplant list and based on clinical outcomes were: 50 cm2/m2 for men and 39 cm2/m2 for women.

Results: IL-6 level was higher in advanced (BCLC C) compared to the early-intermediate (BCLC A-B) stages (21.4 vs 7.7 pg/mL, p< 0.005). On multivariate analysis, IL-6 levels were statistically dependent on the degree of liver disease severity (CP score) and HCC stages (p=0.001, p=0.044, respectively). Sarcopenic patients presented lower BMI (24.7±5.3 vs 28.5±7.0), higher PMN/lymphocyte ratio (2.9±2.4 vs 2.3±1.2) and increased values of log (IL-6) (1.3±0.6 vs 1.1±0.3). Univariate logistic regression between sarcopenia and log (IL-6) showed a significant Odds-ratio (OR 14,88, p=0.044) with an AUC of 0.72.

Conclusion: IL-6 appears an effective biomarker for the diagnosis of advanced cirrhotic HCC. In addition, IL-6 could be considered as a marker of cirrhotic HCC-related sarcopenia when a CT dedicated software is not available.

Figure 1. Contour plot between IL 6, Child Pugh score and HCC stages.

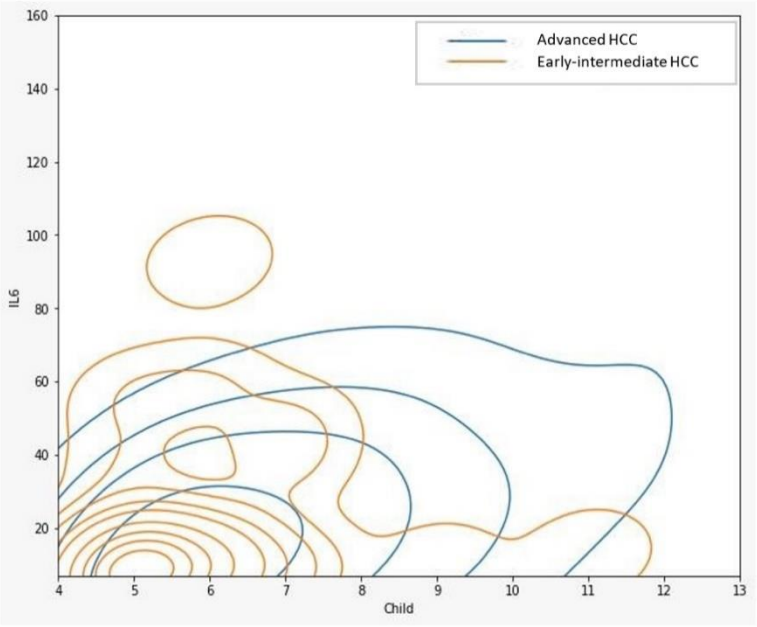

Table 1. Univariate and Multivariate binomial regression between sarcopenia and other variables.
Legend: BMI, body mass index; IL-6, interleukin-6; PMNs, polymorphonucleates; HCC, hepatocellular carcinoma; CP, Child-Pugh.

	Univariate analysis			**Multivariate analysis**		
	estimate	**t**	**p**	**estimate**	**t**	**p**
BMI >30	-0.91	-1.27	0.20	-1.74	-1.85	ns
Log (IL-6)	2.7	2.0	**0.044**	3.63	1.89	**0.05**
PMNs/Lymphocites	0.18	1.42	0.15	0.13	0.79	ns
HCC stages	-0.41	-0.57	0.56	-0.36	-0.41	ns
CP score	-0.38	-0.55	0.58	-0.73	-0.42	ns

P08-17
Impact of surveillance strategies for hepatocellular carcinoma on patients with chronic liver diseases

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Background and Aims: International guidelines suggest that surveillance strategies of patients with chronic liver diseases improve their clinical outcome. The aim of our study is to determine the impact of the strategies of surveillance in patients of a tertiary hospital in Romania.

Method: We performed a prospective study on patients diagnosed with hepatocellular carcinoma during January 2021-December 2022 in Iasi, a university medical center in Romania. We assessed outcomes, based on the adherence to surveillance strategies.

Results: A total of 4785 were admitted in the Gastroenterology and Hepatology Department, 2900 with chronic liver diseases, including decompensated cirrhosis. 100 of new cases of hepatocellular carcinoma were diagnosed, of these 66% were enrolled in surveillance programme. Only 60,6% of patients under surveillance were adherent, which improved their survival.

Conclusion: The national rules of surveillance for hepatocellular carcinoma improved the overall survival and had a favorable impact on shortening the hospitalization of patients.

P08-19
Fib4 can help select cost effective HCC screening populations in Child Pugh A cirrhosis

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Background and Aims: Hepatocellular carcinoma (HCC) represents 90% of primary liver cancers, which were 5% of all global cancer diagnoses in 2020, and the third most common cause of cancer-related death. EASL and AASLD recommend that population screening for HCC is cost effective if annual HCC incidence ≥1.5%. The authors aimed to identify predictors of ten year HCC risk in compensated cirrhosis.

Method: Child Pugh A cirrhosis patients without history of decompensation or HCC and with Transient Elastography (TE) liver stiffness measurement (LSM) ≥12kPa were enrolled between 2011-2013. Baseline bloods were used to calculate Fib4. Follow up was until death or ten years. Primary outcome was development of HCC. Data was analysed using SPSS.

Results: 114 patients were included (61% male). The majority of patients had hepatitis C virus (HCV) (38%) or alcohol related liver disease (30%). Mean age at TE was 53 years (+-13). Median LSM was 21.7kPa (16.5-36.8). 11% of patients developed HCC over the study period. Annual incidence of HCC among the entire cohort was 1.33%.
Higher Fib4 scores were found to associate with increased risk of HCC development (p = 0.001, spearman 0.311). Median Fib4 score was 5.87 (3.97-10.62) in those who developed HCC vs 2.98 (1.9-4.6) in those who did not (p = 0.006). On cox regression analysis, after controlling for gender, age at TE, and aetiology; for every 1 unit increase in Fib4 there is a 9.8% increased 10 year risk of HCC (p = 0.02, HR 1.098, 95% CI 1.015-1.188).
The diagnostic performance of Fib4 for HCC was assessed using receiver operated characteristic curves (ROC). The area under the ROC (AUROC) was 0.79 (95% CI: 0.68-0.9, p = 0.001) and this was used as an index to develop proposed cut offs for enrolment in HCC surveillance programs with results demonstrated in the table below.
Neither index LSM (p = 0.618) nor age at TE (p = 0.239) predicted development of HCC.
On subgroup analysis; 18% of HCV patients developed HCC over the ten year period. Index LSM did not predict HCC (p = 0.870). Older age at TE correlated with higher risk of developing HCC (p = 0.02, pearson 0.339). Higher Fib4 scores correlated with increased risk of HCC (p = 0.011, spearman 0.381). On Cox regression analysis, when controlling for gender and LSM, for each additional year of age at TE, 10 year risk of HCC increased by 9.5% (p = 0.031, HR 1.095, CI 1.008 – 1.189).

Conclusion: This study identifies the potential use of Fib4 as a predictive tool for HCC development in compensated cirrhosis. Our data suggests that introducing a Fib4 lower limit of >1.2 for enrolment in HCC surveillance would improve cost effectiveness by meeting EASL and AASLD guidelines for annual HCC incidence of ≥1.5%. As illustrated, using higher cut offs would create greater cost effectiveness in population screening while maintaining very high sensitivity for HCC development, and could be used for identifying high risk populations.

Figure:

TOOL	CUT OFF	SENS.	SPEC.	PPV	NPV	ANNUAL HCC INCIDENCE INCLUDED POPULATION	% COHORT NOT REQUIRING SURVEILLANCE USING CUT OFF	ANNUAL HCC INCIDENCE EXCLUDED POPULATION
FIB4	>1.2	100%	7%	11%	100%	1.51%	6.3%	0%
	>2.4	100%	39%	16.4%	100%	2.24%	34.8%	0%
	>3.34	92%	62%	22.4%	98.4%	3.3%	56%	0.18%
	>3.82	83%	71%	25.6%	97.2%	3.8%	65.2%	0.32%

P08-20
TKI treatments for HCC before liver transplantation: a collaborative European study

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Background and Aims: Recent advances in systemic treatments for hepatocellular carcinoma (HCC) have prompted the discussion regarding their possible role for downstaging advanced HCC prior to liver transplantation (LT) or for bridging to LT to prevent tumor progression and reduce the dropout risk.

Method: Data of patients with HCC treated with different TKI before LT were collected using an online Survey in 16 different centres across Europe.

Results: 48 patients with a mean age of 60 years and receiving a LT between December 2006 and September 2022 were enrolled. 26 patients were treated with TKI with a downstaging purpose, while 22 received TKI as a bridging treatment to LT. 34 patients (71%) received sorafenib, 13 lenvatinib (27%) and 1 patient (2%) a sequential therapy with sorafenib-regorafenib. 41 patients (85%) received at least one locoregional treatment before LT. 27 patients were Milan-in at listing (56%). After a median follow up of 464 days (20-3005), 6 patients (12.5%) experienced recurrence of HCC after LT (3 patients were Milan-in and 3 Milan out at listing). We observed 4 cases of early mild-moderate graft rejection (8%) and 8 (16%) cases of vascular complications (artery thrombosis or arterial bleeding) early after LT. All vascular complications occurred in the group of patients treated with TKI as downstaging treatment, with last administration of TKI 158 days before LT.

Conclusion: This is largest series of patients receiving TKI pre-LT reported to date which confirms very favorable short-term outcome (93% one-year overall survival).

Figure:

Table 1. Patients Main features (=48 pts)	
Mean Age, (IQR)	60 (44-69)
Underlying liver disease, n (%) - Viral Hepatitis - Alcohol - MAFLD - Other	 - 31 (64.5) - 9 (18.7) - 6 (12.5) - 3 (6.25)
Clinically Significant portal Hypertension with Esophageal Varices, n (%)	10 (21)
Type of donor, n (%) - DBD - DCD - Living donor	 - 37 (77) - 10 (21) - 1 (2)
Alfa-fetoprotein at listing, m (IQR)	193 (1-2986)
Milan criteria at listing, n (%) - Milan in - Milan out	 - 27 (56) - 21 (43)
Patients received at least one locoregional treatments before LT, n (%) - **Resection** - **TACE, TARE, TAE** - **Ablation** - **SRBT**	41 (85%) - 11 (23) - 30 (62.5) - 14 (29) - 2 (4)
Purpose of TKIs treatment - **Bridging** - **Downstaging**	 - 22 (45) - 26 (55)
Type of TKIs, n (%) - **Sorafenib** - **Levantinib** - **Regorafenib**	 - 34 (71) - 13 (27) - 1 (2)
Time from last dose of TKI to LT, m (IQR) - **Bridging group**	 - 21 (0-113)

P09-01
Implication of patients experience in the liver cancer multidisciplinary approach

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Background and Aims: The three pillars of the Quality of Health Care are efficacy, safety and patients experience (PE). Additionally, it has been demonstrated that the multidisciplinary approach is associated to better outcome. The last version of BCLC staging system includes the chapter on '*clinical decision-making'* showing the multiparametric approach used by physicians when selecting treatments including patient perspective **Aim:** to evaluate PE in the BCLC group.

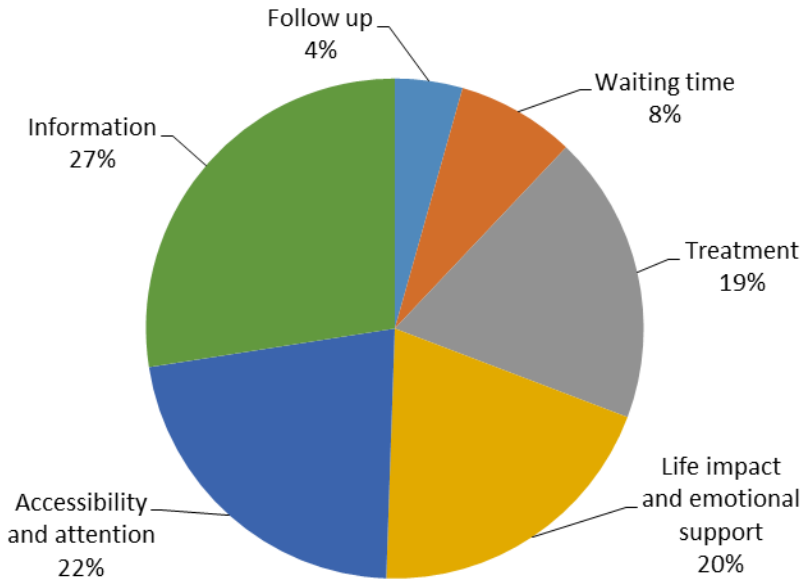
Method: The project was divided into 3 parts: 1) The Patient Experience Team (PExT) and professionals from BCLC map the patient journey, the stakeholder map, and identify the patient's archetype; 2) based on the information from part 1, the PExT designed questions. The BCLC nurses, according the archetypes, invited patients for the focal groups/interviews and 3) PExT did the focal groups/interviews. All the interventions were recorded and verbatim transcription was analyzed through MAXQDA software.

Results: A total of 11 patients and 3 caregivers of patients who died due to liver cancer were invited. Eleven patients participated in 3 focal groups and 3 caregivers were interviewed. In the focal groups 91 concepts were identified and were grouped into 23 categories and 6 meta-categories (Figure 1). The most frequent meta-categories were related to the information received, the contact with professionals, the impact of cancer in their life and the support received. The majority of patients did not need to look further information on the internet, they found that the information at diagnosis was clear, precise and enough but they would like to have more time to clarify doubts. The patients were aware of the role of the different professionals and saw them as a coordinated group but some of them requested more information regarding the 'clinical decision process.' The majority of patients agreed that the diagnosis had affected their lives and most of them commented that the best support was their family despite of the fact that some received external support. In the caregivers interviews the good coordination between the Hospital and palliative home care teams was mentioned. However, they raised the difficulties that they had to openly speak about death with their relatives due to the cultural barrier and the need of support for caregivers in this regard. The PE can be positive even in situations where outcomes worsen, such as in the case of the end of life.

Conclusion: Patients and caregivers are interested on aspects related to contact with professionals and the support received. Thus, the evaluation of PE helps the BCLC team to promote Value-based Heath Care. In addition, this study reinforces the need of information given during the follow-up visits or

organizing activities not only with patients but also for caregivers as well as developing the PROMs/PREMs to effectively implement ‘shared-decision making’.

Figure:



P09-03-YI
Comparison between Regular Additional Endobiliary Radiofrequency Ablation and Photodynamic Therapy in Patients with Advanced Extrahepatic Cholangiocarcinoma under Systemic Chemotherapy

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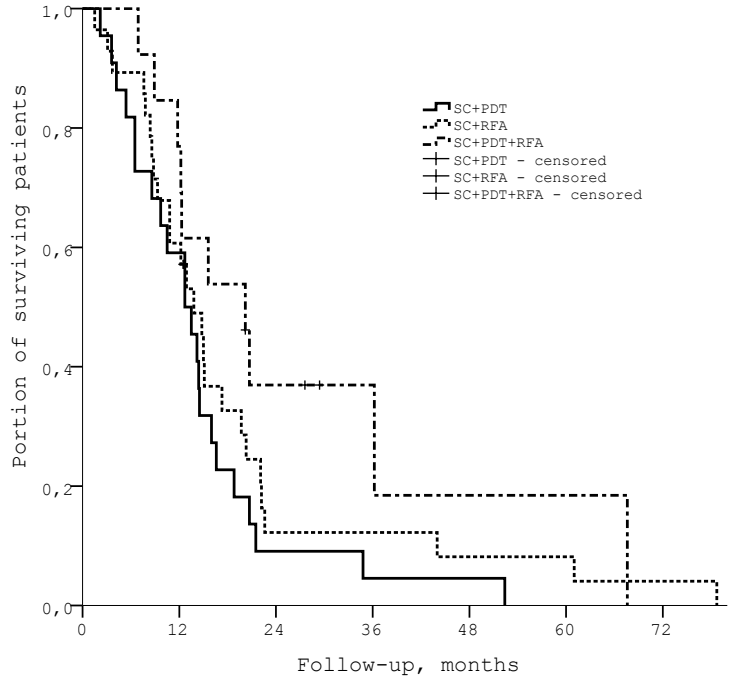
Background and Aims: Extrahepatic cholangiocarcinoma remains a malignancy with a dismissal prognosis. The palliative therapy standard consists out of palliative systemic chemotherapy (SC) and biliary drainage through stenting. Endobiliary ablative techniques like photodynamic therapy (PDT) and radiofrequency ablation (RFA) have demonstrated feasibility and beneficial data on survival. This study aimed to examine the overall survival, progression-free survival and treatment associated adverse events in patients treated with systemic chemotherapy and concomitant endobiliary ablation.

Method: All patients with extrahepatic cholangiocarcinoma treated in the University Hospital Bonn between 2006 and 2021 were evaluated for study inclusion. 63 patients received a combination of systemic chemotherapy and at least one endobiliary ablative treatment and were retrospectively analyzed.

Results: Patients were stratified into three groups: SC + ePDT (n = 22), SC + eRFA (n = 28) and SC + ePDT + eRFA (n = 13). Baseline characteristics were well balanced between the groups. The median OS of the whole cohort was 14.2 months (95% CI: 12.0 – 16.4) with no statistically significant difference between the three therapy groups but a trend to better survival for the triple therapy group (ePDT + SC: 12.7 months (95% CI: 8.4 – 17.0); eRFA + SC: 13.8 months (95% CI: 9.8 – 17.8); ePDT + eRFA + SC: 20.2 months (95% CI: 11.0 – 29.4); p = 0.112). The same constellation was observed for the progression-free survival (ePDT + SC: 6.4 months; eRFA+SC: 8.0 months; ePDT + eRFA + SC: 6.7 months; p = 0.662). In the multivariate cox regression analysis eRFA (HR 0.44, 95% CI: 0.25 – 0.80), more than one line of SC (HR 0.48, 95% CI: 0.26 – 0.88) and metal stent placement (HR 0.33, 95% CI: 0.17 – 0.62) remained independent predictors of survival. Overall, therapy was well tolerated and showed no differences between the groups. Merely, cholangitis occurred more often within the SC + eRFA group.

Conclusion: Additional endobiliary ablative therapies in combination with systemic chemotherapy was feasible and well tolerated. Both modalities, eRFA and ePDT showed similar benefit in terms of survival. However, in the eRFA group more cholangitis seems to occur. Interestingly, patients receiving both regimes showed the best overall survival indicating a possible synergism between both ablative therapies. Multivariate analysis highlighted a beneficial effect through eRFA. Prospective trials comparing these therapy regimes in combination with standard systemic therapy are urgently needed.

Figure:


1,0
0,8
0,6
0,4
0,2
0,0
Portion of surviving patients
0
12
24
36
48
60
72
Follow-up, months
SC+PDT
SC+RFA
SC+PDT+RFA
SC+PDT - censored
SC+RFA - censored
SC+PDT+RFA - censored

P09-05-YI
Prognostic impact of metastatic site in patients receiving first-line sorafenib therapy for advanced hepatocellular carcinoma

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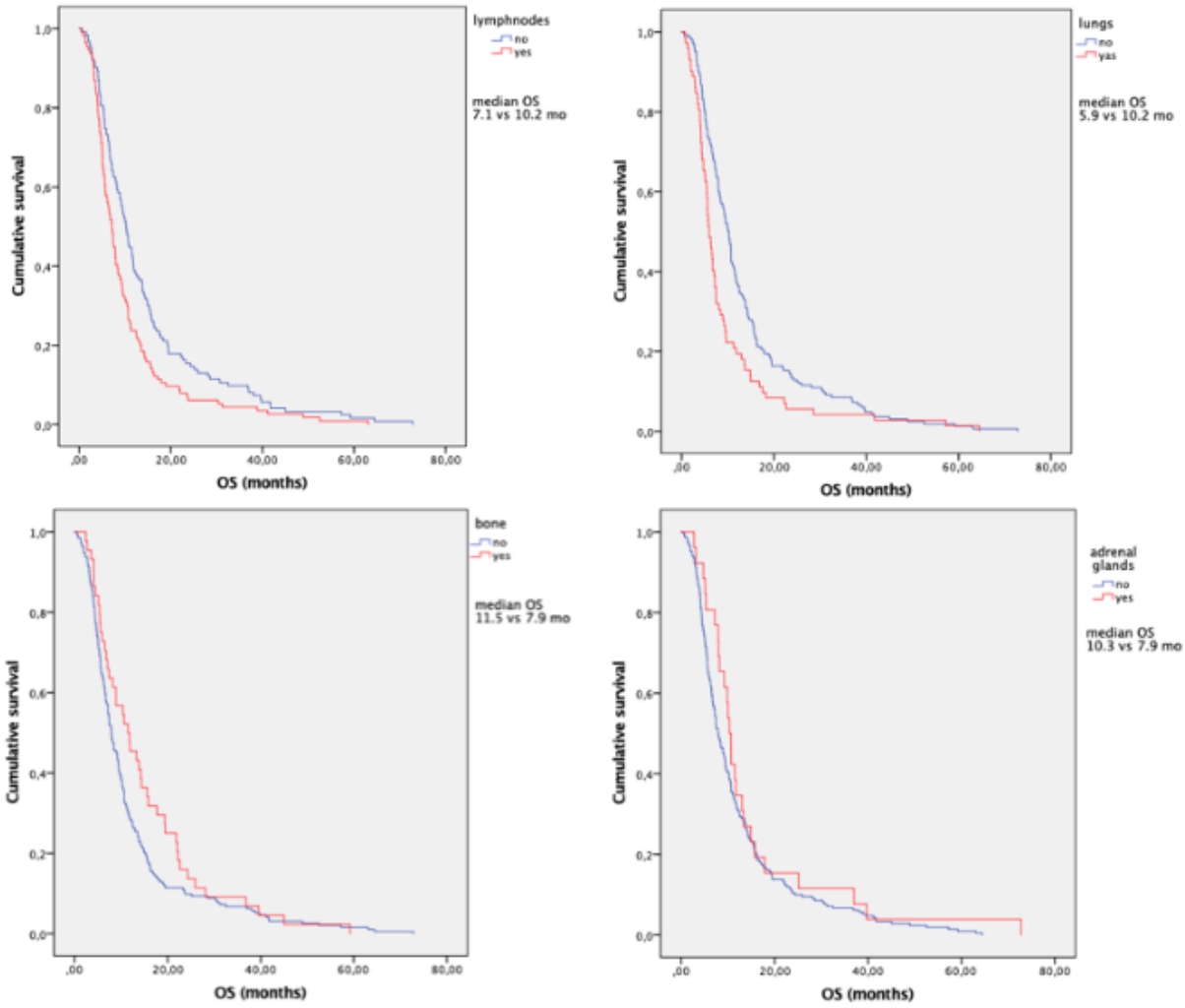
Background and Aims: Extrahepatic spread is a well-known negative prognostic factor in patients with advanced hepatocellular carcinoma (HCC). The prognostic role of different metastatic sites and their response rate to systemic treatment is still being debated.

Method: We considered 237 metastatic HCC patients treated with sorafenib as first-line therapy in five different Italian centers from 2010 to 2020.

Results: The most common metastatic sites were lymph nodes, lungs, bone and adrenal glands. In survival analysis, the presence of dissemination to lymph nodes (OS 7.1 vs 10.2 months; p = 0.007) and lungs (OS 5.9 vs 40 10.2 months; p <0.001) were significantly related to worse survival rates compared with all other sites. In the subgroup analysis of patients with only a single metastatic site, this prognostic effect remained statistically significant. Palliative radiation therapy on bone metastases significantly prolonged survival in this cohort of patients (OS 19.4 vs 6.5 months; p <0.001). Furthermore, patients with lymph node and lung metastases had worse disease control rates (39.4% and 30.5%, respectively) and shorter radiological progression-free survival (3.4 and 3.1 months, respectively).

Conclusion: In conclusion, some sites of extrahepatic spread of HCC have a prognostic impact on survival in patients treated with sorafenib; in particular, lymph node and lung metastases have worse prognosis and treatment response rate.

Figure:


lymphnodes
no
yes
median OS
7.1 vs 10.2 mo
Cumulative survival
OS (months)
lungs
no
yes
median OS
5.9 vs 10.2 mo
Cumulative survival
OS (months)
bone
no
yes
median OS
11.5 vs 7.9 mo
Cumulative survival
OS (months)
adrenal glands
no
yes
median OS
10.3 vs 7.9 mo
Cumulative survival
OS (months)

P09-07
Trial in progress: An open-label, multicenter study investigating RP3 oncolytic immunotherapy in combination with first- or second-line systemic atezolizumab and bevacizumab in locally advanced unresectable or metastatic hepatocellular carcinoma

Adel Samson[1], Tanios Bekaii-Saab[2], Mark Yarchoan[3], Muneeb Ahmed[4], Fouzia Boulhabel[5], David Cohan[5], Jaroslaw Jac[5], Wen Wee Ma[6]

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Background and Aims: Despite advances in treatment for unresectable hepatocellular carcinoma (HCC), long-term survival rates remain poor. The combination of bevacizumab (Bev) and atezolizumab (Atezo) is the preferred frontline therapy for advanced HCC, but a minority of patients (pts) respond, and secondary resistance usually occurs within months. HCC has an immune-suppressed tumor microenvironment, mediated by activated immune checkpoint signaling and angiogenesis pathways, which may contribute to therapeutic resistance. RP3 is a genetically modified herpes simplex virus type 1 (HSV-1) that expresses the fusogenic gibbon ape leukemia virus glycoprotein with the R sequence deleted (GALV-GP-R–), an anti–CTLA-4 antibody-like molecule, CD40 ligand, and 4-1BB ligand. The direct oncolytic effect coupled with immune stimulation by RP3 in the tumor microenvironment is intended to provide systemic antitumor activity and enhance therapeutic response to anti–PD-1/PD-L1 agents, such as Atezo. Preclinical data have demonstrated improved distribution of oncolytic HSV within tumors in combination with Bev, supporting the clinical combination of RP3 with Bev. This study will evaluate the safety and efficacy of RP3 combined with Atezo and Bev as first- (1L) and second-line (2L) systemic therapies for unresectable and advanced HCC.

Method: The 1L and 2L cohorts will each enroll up to 30 pts. Pts in the 1L cohort may not have received prior systemic treatment; pts in the 2L cohort must have progressed on or after 1 prior line of systemic therapy, which must have included a PD-1/PD-L1−directed agent. Key inclusion criteria include advanced, unresectable HCC with ≥1 measurable tumor of ≥1 cm in longest diameter, Child-Pugh Class A, and Eastern Cooperative Oncology Group performance status of 0−1. Key exclusion criteria include untreated esophageal and/or gastric varices with bleeding or at high risk for bleeding and macroscopic invasion of tumor into any major blood vessel(s) and/or main bile ducts. Pts with a history of medically refractory hepatic encephalopathy and/or hepatorenal syndrome are also excluded. Pts in the 1L cohort will receive 1200 mg Atezo and 15 mg/kg Bev every 3 weeks (Q3W) together with RP3 intratumorally Q3W for a total of up to 8 doses. Pts in the 2L cohort will receive RP3 every 2 weeks for 4 doses with Bev Q3W starting on cycle (C) 1 day (D) 1, then RP3 and Bev Q3W for up to 4 more doses, with Atezo Q3W being added on C4D1. The primary endpoint is overall response rate (ORR) by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. Secondary endpoints are safety, ORR using HCC modified RECIST, duration of response, complete response rate, and progression-free survival.

P09-09
Impact of PNPLA3 rs738409 polymorphism on the development of hepatocellular carcinoma in female patients with non-alcoholic fatty liver disease and severe liver fibrosis

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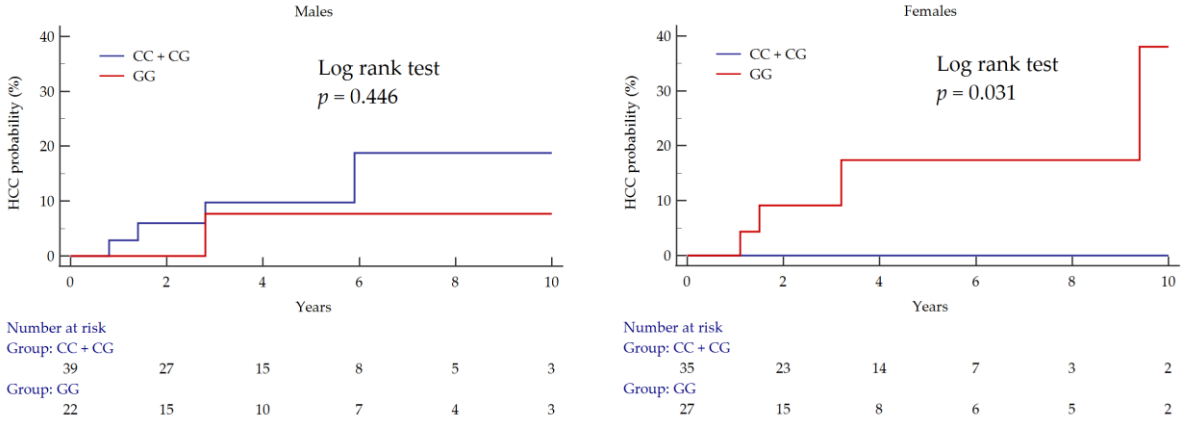
Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is a complex trait, resulting from the interplay between environmental determinants and genetic variations. The rs738409 single nucleotide polymorphism (SNP) C > G in the patatin-like phospholipase domain-containing 3 (*PNPLA3*) gene accounts for the largest fraction of genetic variability of hepatic fat accumulation. In patients with NAFLD, the GG variant is associated with higher risk of liver disease progression. Here, we aimed to identify subgroups of individuals in which the *PNPLA3* SNP has a stronger impact on the development of HCC.

Method: We retrospectively enrolled 123 patients (mean age 58 years [IQR = 50 - 67]; 61 males [49.6 %]; median BMI 31 kg/m^2 [26.8 - 33.2]) with severe hepatic fibrosis (F = 3: N = 51 [41.5 %]) or clinical diagnosis of NAFLD-related liver cirrhosis (F = 4: N = 72 [58.5 %]). All patients had at least 6 months FU with regular US surveillance (annually in F2 and biannually in F3/F4). Genotyping for *PNPLA3* SNP rs738409 was performed by real-time allelic discrimination assay (TaqMan SNP Genotyping Assay, Applied Biosystems).

Results: Overall, the *PNPLA3* rs738409 genotype was CC in 25, CG in 49 and GG in 49 patients (20.3 %, 39.8 % and 39.8 %, respectively). After a median follow-up of 3.2y (IQR = 1.5 - 5.9), 10 patients developed HCC (8.1 %; incidence rate: 1.9 per 100 person/years). Notably, 9 out of 10 HCC occurred in patients with liver cirrhosis, while only one HCC occurred in F3 patients. Among patients who developed HCC, the *PNPLA3* CC, CG and GG genotypes were present in 1 (10 %), 3 (30 %) and 6 (60 %) cases. While in males we did not observe a significant difference in HCC occurrence according to *PNPLA3* genotype (CC+CG 4/39 [10.3 %] vs. GG 2/22 [9.1 %]; Log rank test, p = 0.446), the cumulative incidence of HCC was significantly higher in females carrying the GG genotype as compared to those carrying the wild type allele (CC+CG 0/35 [0 %] vs. GG 4/27 [14.8 %]; Log rank test, p = 0.031) (Figure 1).

Conclusion: NAFLD females carrying the *PNPLA3* GG risk genotype are at higher risk of developing HCC compared to those carrying the wild type allele (CC/CG). *PNPLA3* genotyping may be a useful tool for the personalization of HCC surveillance in NAFLD patients at risk.
This work has received support from EU/EFPIA/IM2 Joint Undertaking (LITMUS grant no.777377)

Figure:


Males
HCC probability (%)
CC + CG
GG
Log rank test
p = 0.446
Years
Number at risk
Group: CC + CG
39 27 15 8 5 3
Group: GG
22 15 10 7 4 3
Females
HCC probability (%)
CC + CG
GG
Log rank test
p = 0.031
Years
Number at risk
Group: CC + CG
35 23 14 7 3 2
Group: GG
27 15 8 6 5 2

P09-11
Atezolizumab/Bevacizumab combination therapy seems to benefit mainly BCLC-C cirrhotic patients with hepatocellular carcinoma who migrate to this stage after disease recurrence following resection or radiofrequency ablation

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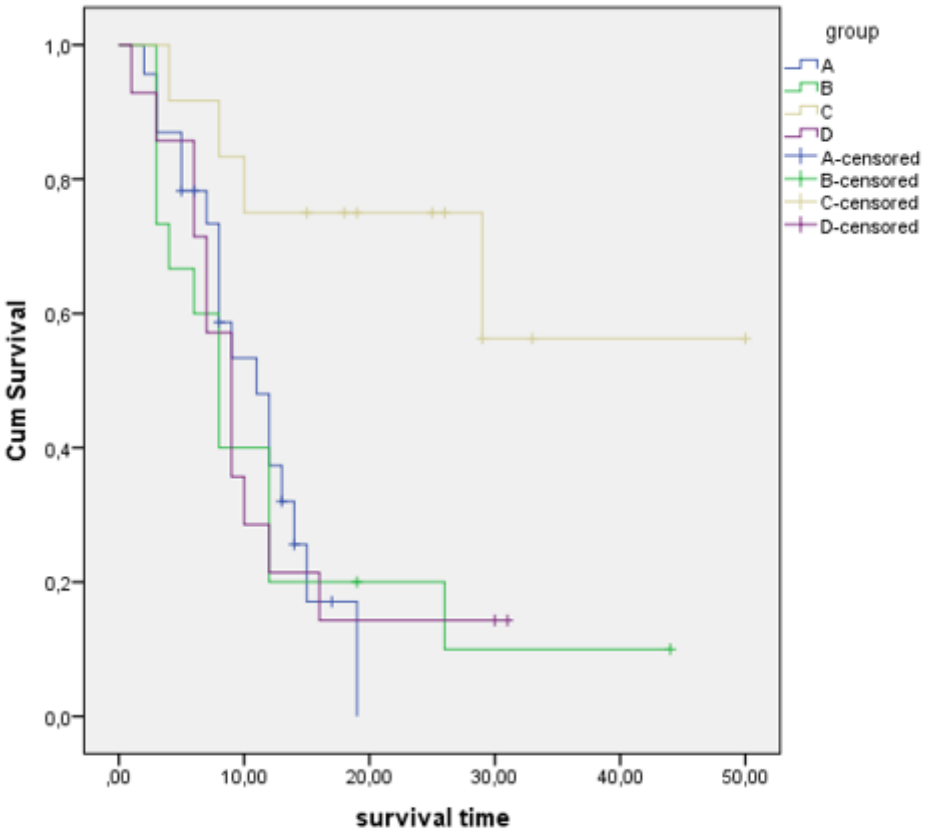
Background and Aims: Registrational study (IM BRAVE 150) mainly included hepatocellular carcinoma (HCC) patients at BCLC-C stage, irrespective of the type of progression to this stage, with well compensated liver disease (CPT-A stage). The aim of our study was to retrospectively evaluate, under real life conditions, the overall survival (OS) of patients with advanced HCC (BCLC-C stage), either initially presenting in the advanced stage or migrating from BCLC-A to BCLC-C stage within 2 years after curative LR or RFA, treated either with atezolizumab-bevacizumab (ATZ/BEV) combination or with TKIs sequentially (sorafenib as 1st line and cabozantinib as 2nd line treatment).

Method: Sixty four cirrhotic patients with advanced HCC (56 males, mean age 67y, 22 with diabetes, CPT-A=45/B=19, mean MELD/Na=11, ALBI grade I=20/grade II=38, 28 with varices, 18 with extrahepatic metastasis, 24 with macrovascular invasion) who either initially presented on the BCLC-C stage and were treated with ATZ/BEV (group A, N=23) or TKIs (group B, N=15) or who migrated from BCLC-A to BCLC-C stage within 2 years after LR or RFA and were treated with ATZ/BEV (group C, N=12) or TKIs (group D, N=14) were evaluated.

Results: The four groups were comparable for all the baseline parameters evaluated (age p=0.9, gender p=0.08, platelets p=0.246, chronic liver disease etiology p=0.408, coexistence of diabetes p=0.314, presence of varices p=0.066, CPT stage p=0.067, ALBI grade p=0.398) except for CPT score (p=0.012) and MELD/Na score (p=0.002). As expected, median OS was significantly higher for group C patients (61m) compared to group D (27m), group A (11m) and group B (8m) patients (p<0,001). Moreover, post-recurrence survival for group C patients seems to be significantly higher compared to those of group D (9m) patients (p=0.007) as well as for group A and B (figure). Using cox regression analysis, we observe that the OS of group A (HR=3.71, 1.20-11.46, p=0.02) was significantly worse than post-recurrence survival of group C, which was comparable to group D (HR=3.14, 0.95-10.35 p=0.06), adjusted for CPT, ALBI and MELD/Na score (figure).

Conclusion: Median survival of ATZ/BEV or TKI-treated patients who were initially classified in BCLC-C stage is less than 12m, irrespective of treatment schedule, as was post-recurrence survival of sequentially TKI-treated patients. ATZ/BEV therapy seems to benefit mainly BCLC-C patients who migrate from earlier stages after curative LR or RFA compared to patients initially presented in the advanced stage. Liver disease severity assessed using CPT and MELD/Na scores seems to drive the overall as well as post-recurrence survival.

Figure: Survival of group A (blue), B (green), C (yellow) and D (purple) and cox regression analysis for survival after the beginning of systemic therapy comparing group C to the other groups and adjusted for CPT, ALBI and MELD/Na scores.



Variables	Hazard ratio	95% confidence interval	P-value
Child number	1,03	0,69 to 1,52	0,89
Meld-Na	1,13	0,98 to 1,31	0,09
ALBI	0,86	0,47 to 1,57	0,62
A vs. C	**3,71**	**1,20 to 11,46**	**0,02**
B vs. C	2,89	0,87 to 9,56	0,08
D vs. C	3,14	0,95 to 10,35	0,06

P09-13
TARDBP Is a Prognostic Biomarker and Correlates With Immune Infiltrates in Liver hepatocellular carcinoma

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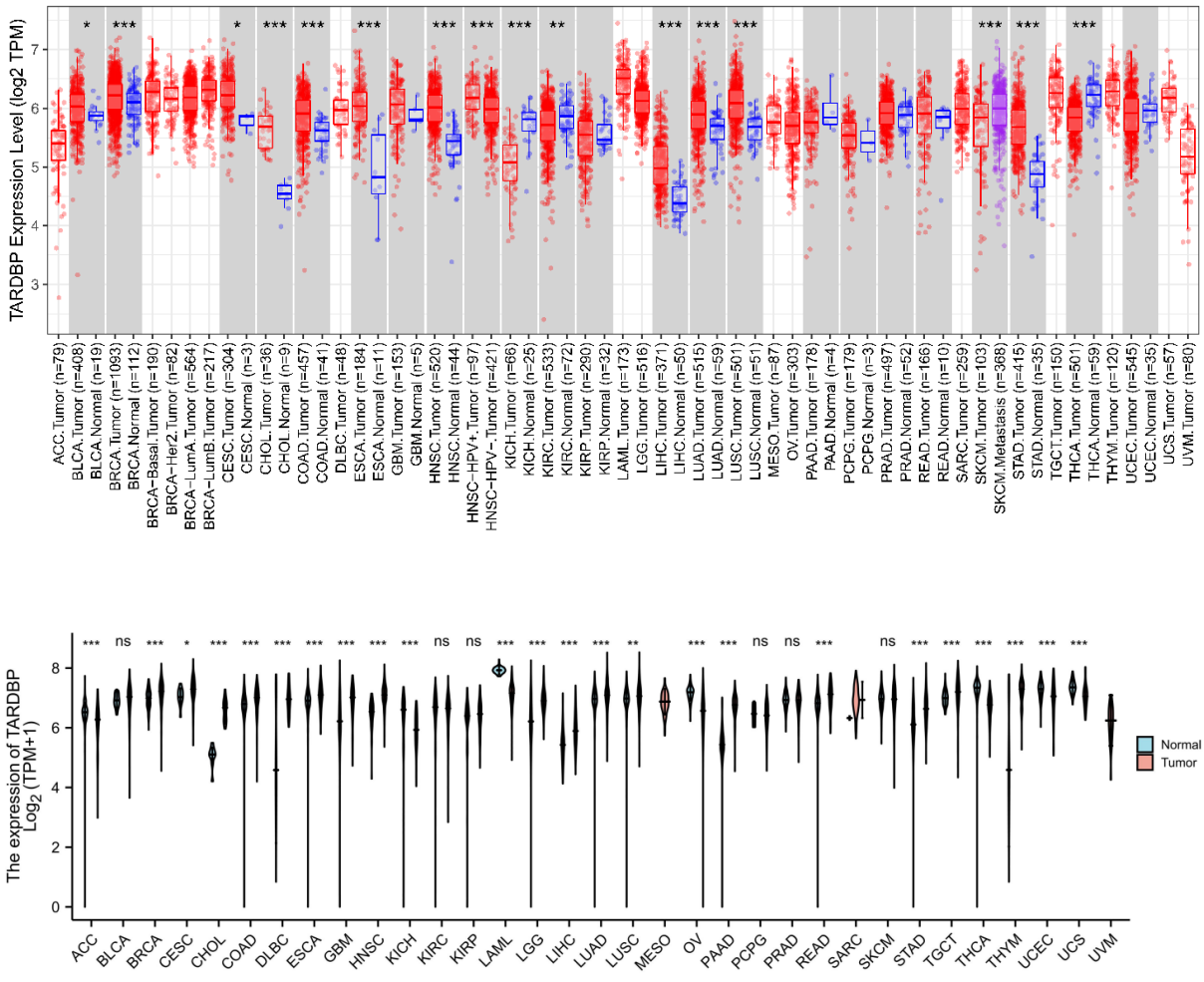
Background and Aims: Transactive response (TAR) DNA-binding protein (TARDBP), also known as TDP-43, has been well studied in neurodegenerative diseases. However, its role in liver hepatocellular carcinoma (LIHC) remains unclear.

Method: The original data of tumors and normal tissues were downloaded from TCGA database and the Genotype-Tissue Expression (GTEx) database through UCSC XENA and use R 3.6.3 software for data analysis. The CPTAC (clinical proteomic tumor analysis consortium) in UALCAN tool was used to analyse protein expression, and Immunohistochemistry (IHC) images were downloaded from HPA website. The receiver operating characteristic (ROC) curve was used to assess the diagnostic value of TARDBP in LIHC. The Kaplan-Meier plots were used to evaluate the correlation between TARDBP expression and prognosis.The correlations between TARDBP and cancer immune characteristics were analyzed via the R 3.6.3 software and TISIDB databases.

Results: TARDBP expression differs significantly in most cancers. Besides, high accuracy in diagnosis LIHC and notable correlations with prognosis of certain cancers ()suggest that TARDBP might be a potential diagnostic and prognostic biomarker of LIHC. We also found that TARDBP expression was correlated with many immune characteristics in LIHC. TARDBP expression was positively correlated with infiltrating levels of aDC, TFH, NK CD56bright cells, T helper cells and Th2 cells. In contrast, the expression level of TARDBP was negatively correlated with the infiltrating levels of DC, Cylotoxic cells, pDC, Tgd, Mast cells and NK cells. Besides, TARDBP expression is correlated with a host of chemokines/chemokine receptors and immunoinhibitors/immunostimulators in LIHC.

Conclusion: TARDBP is a remarkable molecular biomarker for diagnosis and prognosis in LIHC. Besides, it can be used as a promissing biomarker for determining the immune infiltration characteristics in LIHC, and could be a new target for immunotherapy.

Figure:


TARDBP Expression Level (log2 TPM)
ACC.Tumor (n=79)
BLCA.Tumor (n=408)
BLCA.Normal (n=19)
BRCA.Tumor (n=1093)
BRCA.Normal (n=112)
BRCA−Basal.Tumor (n=190)
BRCA−Her2.Tumor (n=82)
BRCA−LumA.Tumor (n=564)
BRCA−LumB.Tumor (n=217)
CESC.Tumor (n=304)
CESC.Normal (n=3)
CHOL.Tumor (n=36)
CHOL.Normal (n=9)
COAD.Tumor (n=457)
COAD.Normal (n=41)
DLBC.Tumor (n=48)
ESCA.Tumor (n=184)
ESCA.Normal (n=11)
GBM.Tumor (n=153)
GBM.Normal (n=5)
HNSC.Tumor (n=520)
HNSC.Normal (n=44)
HNSC−HPV+.Tumor (n=97)
HNSC−HPV−.Tumor (n=421)
KICH.Tumor (n=66)
KICH.Normal (n=25)
KIRC.Tumor (n=533)
KIRC.Normal (n=72)
KIRP.Tumor (n=290)
KIRP.Normal (n=32)
LAML.Tumor (n=173)
LGG.Tumor (n=516)
LIHC.Tumor (n=371)
LIHC.Normal (n=50)
LUAD.Tumor (n=515)
LUAD.Normal (n=59)
LUSC.Tumor (n=501)
LUSC.Normal (n=51)
MESO.Tumor (n=87)
OV.Tumor (n=303)
PAAD.Tumor (n=178)
PAAD.Normal (n=4)
PCPG.Tumor (n=179)
PCPG.Normal (n=3)
PRAD.Tumor (n=497)
PRAD.Normal (n=52)
READ.Tumor (n=166)
READ.Normal (n=10)
SARC.Tumor (n=259)
SKCM.Tumor (n=103)
SKCM.Metastasis (n=368)
STAD.Tumor (n=415)
STAD.Normal (n=35)
TGCT.Tumor (n=150)
THCA.Tumor (n=501)
THCA.Normal (n=59)
THYM.Tumor (n=120)
UCEC.Tumor (n=545)
UCEC.Normal (n=35)
UCS.Tumor (n=57)
UVM.Tumor (n=80)

The expression of TARDBP
Log2 (TPM+1)
Normal
Tumor
ACC
BLCA
BRCA
CESC
CHOL
COAD
DLBC
ESCA
GBM
HNSC
KICH
KIRC
KIRP
LAML
LGG
LIHC
LUAD
LUSC
MESO
OV
PAAD
PCPG
PRAD
READ
SARC
SKCM
STAD
TGCT
THCA
THYM
UCEC
UCS
UVM

Figure1. Expression level of TARDBP gene in tumors and normal tissues. (A) TARDBP expression in TCGA tumors and adjacent normal tissues; (B) TARDBP expression in TCGA tumors and normal tissues with the data of the GTEx database as controls (*p<0.05, **p< 0.01, ***p<0.001).

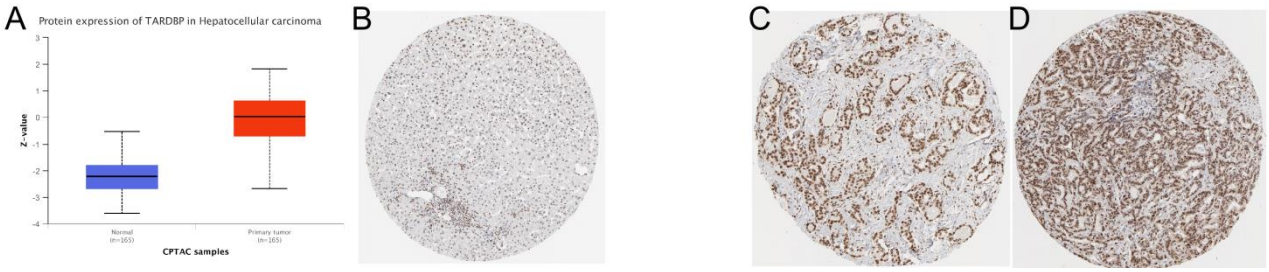


Figure 2. (A) The protein level of TARDBP in tumors . (B-D) The IHC images of LIHC

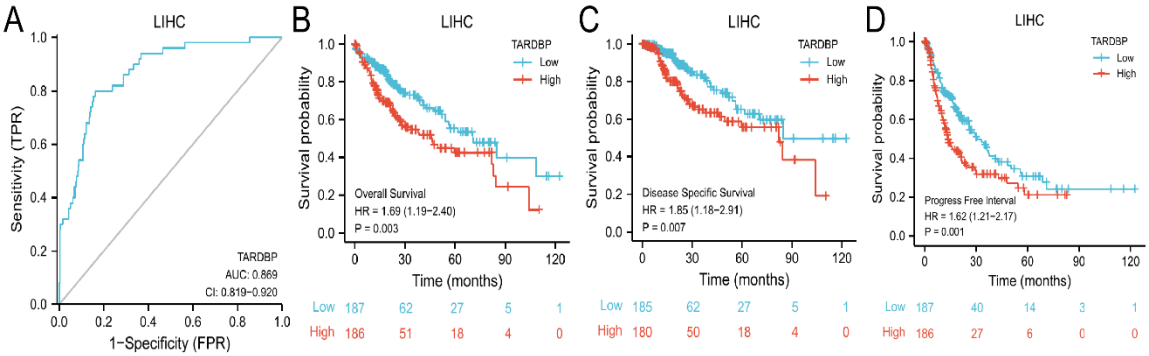


Figure 3. (A) Receiver operating characteristic (ROC) curve for TARDBP expression in LIHC. (B-D) Correlations between TARDBP expression and the prognosis (OS, DSS, and PFI) of LIHC.

P09-15
Single-cell RNA sequencing data reveal cellular landscape and cellular communication for programmed cell death-1 (PD-1)-targeted therapy in patients with hepatocellular carcinoma

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Background and Aims: In hepatocellular carcinoma, PD-1/PD-L1 inhibitors kill tumors by acting on immune cells. But the efficacy is still not satisfactory. The objective of this study was to explore the cellular landscape and cellular communication outcomes of PD-1/ pd-l1 targeted therapy by single-cell sequencing observation.

Method: We used single-cell sequencing to analyze single nucleated cells from multiple human hepatocellular carcinoma tissue samples and paracancer tumor tissue samples. The immune profiles of different treatment responses and different samples were systematically analyzed and statistically compared.

Results: The proportion of effector T cells in CD8+ was significantly decreased, while the proportion of Treg cells in CD4+T cells was significantly increased. Treg group showed significant enrichment in NF-Kappa B, TNF, T cell receptor and other corresponding signaling pathways. At the same time, the expression level of PDCD1 was lower than that of CTLA4,TIGIT,HAVCR2 and other immune checkpoint genes, which were mainly expressed in Treg cell population.

Conclusion: We found significant heterogeneity of immune cells and differences in patient gene expression in patients with different therapeutic effects of PD-1 inhibitors.

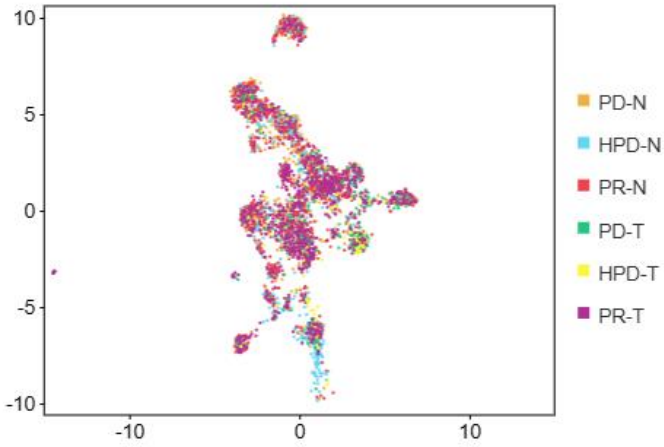

Figure1. UMAP of T cells in different therapeutic groups and different tissues.

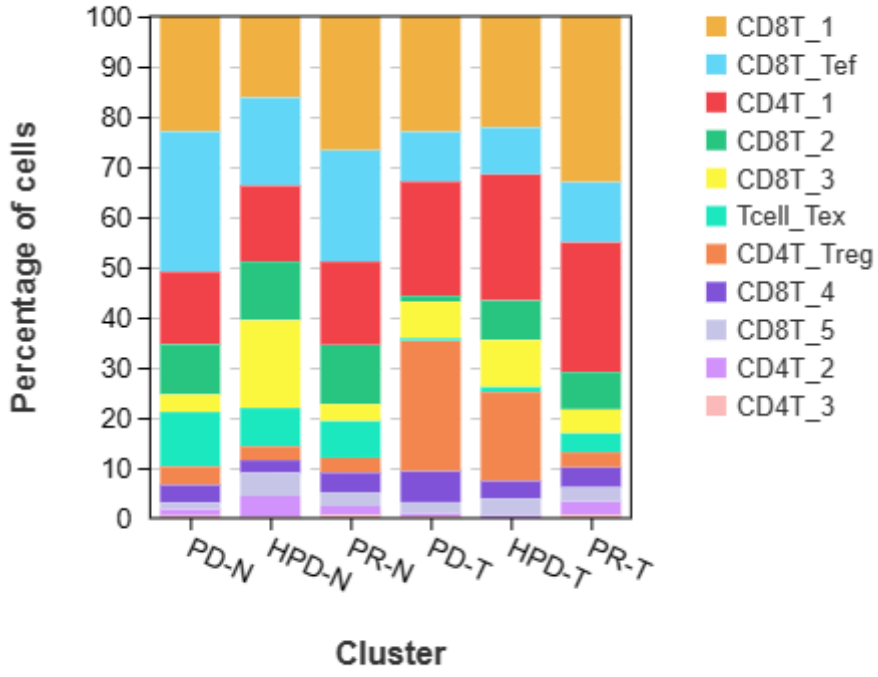

Figure2. Percentage of T cells in different therapeutic groups and different tissues.

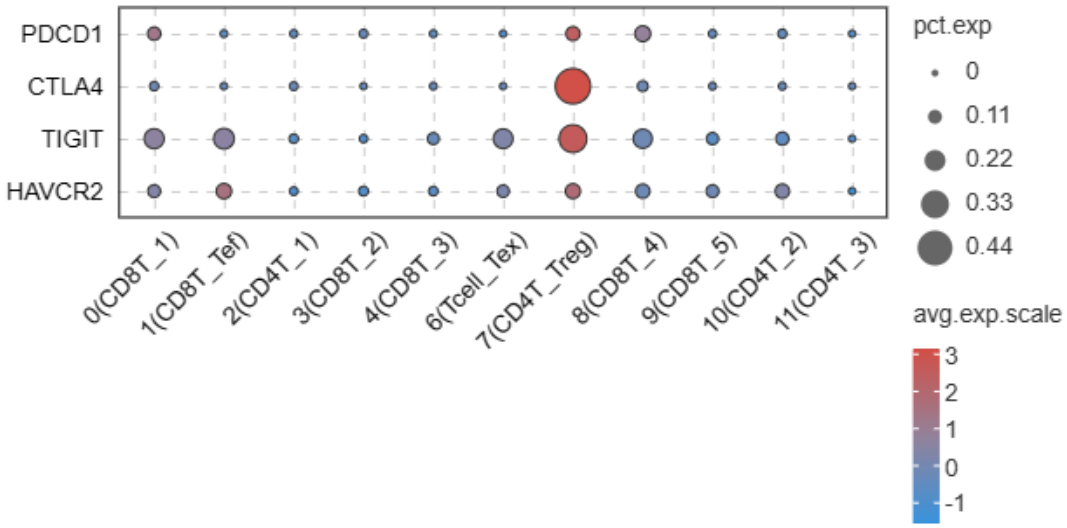

Figure3. Expression of different immune checkpoint genes in T cell clusters.

P09-17
Insulin-like growth factor-binding proteins 2 and 3 and the occurrence, characteristics, and outcome of hepatitis C-related hepatocellular carcinoma: A case-control study

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Background and Aims: This work aimed to study the relation between Insulin-like growth factor-binding proteins-2 and 3 (IGFBP-2, IGFBP-3) and the development, characteristics, and outcome of chronic hepatitis-C (CHC)-related hepatocellular carcinoma (HCC)

Method: This case-control study included patients with CHC with and without HCC (15th of August-2018 till 16th of November-2019) and followed for 18 months. Baseline serum IGFBP-2 and IGFBP-3 were measured using ELISA. We studied the patients and tumor characteristics, treatment, and overall survival.

Results: This study included 82 patients with HCC and 81 non-HCC patients. Twenty-Five (30.5%) patients developed HCC following direct acting antivirals (DAAs) therapy. IGFBP-2 was significantly higher in patients with HCC compared to non-HCC (440.49 (93.60- 729.17)ng/ml versus 184.00 (138.00-284.00)ng/ml respectively, P-value<0.001). IGFBP-3 was significantly lower in patients with HCC (836.45 (733.00- 1137.00)ng/ml versus 1702.70 (836.50- 3141.40)ng/ml respectively, P-value<0.001)**.** No difference found in IGFBP-2, IGFBP-3 levels regarding previous DAAs therapy.

The independent predictors of HCC development are age (OR=1.291, 95%CI=1.163-1.432, P-value<0.001), liver stiffness measurements (LSM) (OR=1.125, 95%CI=1.056-1.199 P-value<0.001), IGFBP-2 (OR=1.002, 95%CI=1.001-1.003, P-value=0.002), IGFBP-3 (OR=0.998, 95%CI=0.997-0.999, P-value=0.001). The independent predictors of mortality are IGFBP-3 (OR=1.002, 95%CI=1.000-1.003), age (OR=0.876, 95% CI=0.808-0.950) and tumor size (OR=1.246, 95% CI=1.002-1.549).

IGFBP-2 is inversely correlated with weight, hemoglobin, albumin and positively correlated with AFP. IGFBP-3 inversely correlated with albumin and positively correlated with platelets, ALT, AST, AFP, Child-Pugh, tumor size, and LSM. IGFBP-3 significantly differed between early and late HCC.

Conclusion: These results provide a correlation between IGFBP-2 and IGFBP-3 levels and CHC-related HCC development and preogression. IGF-BP-2 and 3 are not affected with previous DAAs.

Figure:

Logistic regression analysis for the independent predictors of the development of HCC and mortality

Predictors of the development of HCC				
	P value	**Odds Ratio**	**95% Confidence interval**	
			Lower	**Upper**
IGFBP-2	0.002	1.002	1.001	1.003
IGFBP-3	0.001	0.998	0.997	0.999
Age (years)	< 0.001	1.291	1.163	1.432
liver Stiffness measurements	< 0.001	1.125	1.056	1.199
Predictors of mortality				
IGFBP-3	0.009	1.002	1.000	1.003
Age (years)	0.001	0.876	0.808	0.950
Focal lesion size or size of largest lesion	0.048	1.246	1.002	1.549

P09-18
Immune checkpoint inhibitor treatment has the potential to stabilize liver function in patients with hepatocellular carcinoma

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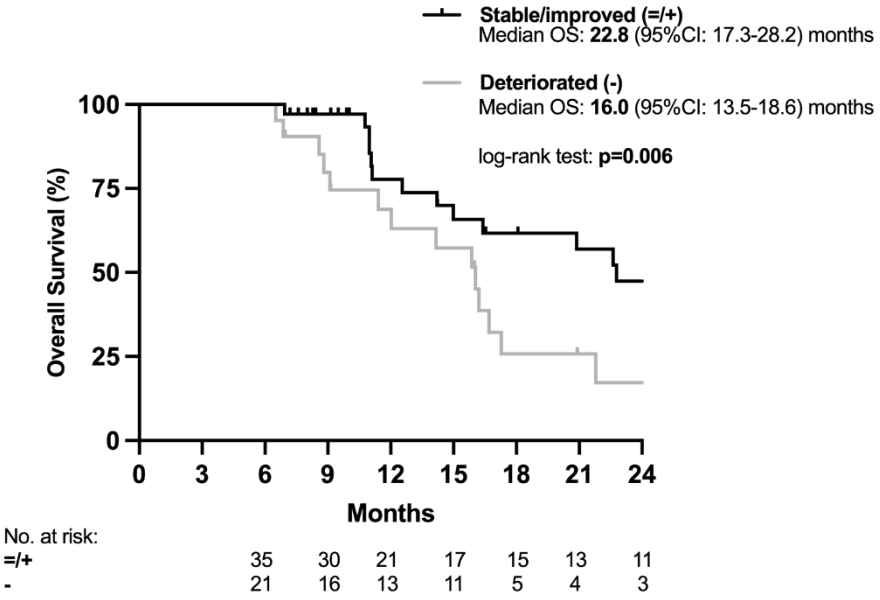
Background and Aims: Deterioration of liver function is a leading cause of death in patients with advanced hepatocellular carcinoma (HCC). Effective anti-cancer treatment may stabilize or even improve liver function. We aimed to evaluate changes in liver function during immune checkpoint inhibitor (ICI) treatment according to radiological response in HCC patients.

Method: HCC patients receiving ICI-treatment between 06/2016-01/2023 at the Medical University of Vienna were included. Liver function was evaluated at the start of ICI-treatment (baseline, BL) as well as 3 and 6 months thereafter using Child-Pugh score (CPS). Any changes in CPS points were defined as deterioration (-) or improvement (+), while equal CPS points were defined as stable (=). Landmark analyses at 3 and 6 months were used to compare overall survival (OS) of patients with improved or stable (+/=) versus deteriorated liver function (-).

Results: Overall, 87 patients (67.6±11.8 years; male: n=64, 74%) were included, of which 64 (74%) had cirrhosis. At BL, median CPS was 6 (IQR: 5-7; CPS-A: 65, 75%; CPS-B: 22, 25%). Three months after ICI initiation, liver function improved or stabilized in 41 (47%) patients according to CPS, while it deteriorated in n=32 (37%) patients and 14 (16%) patients died. Comparable results were observed at 6 months: while 25 (29%) patients died and 6 (7%) patients were lost-to-FU, an improvement/stabilization of CPS was achieved in 35 (40%) patients, compared to 21 (24%) patients with deterioration of CPS. Improvement/stabilization of CPS at 3 and 6 months was associated with a significantly better overall survival (OS) following landmark analysis (at 3 months: +/=: 17.3 (95%CI: 9.8-24.8) vs. -: 12.6 (95%CI: 8.1-17.0) months, p=0.042; at 6 months: +/=: 22.8 (95%CI: 17.3-28.2) vs. -: 16.0 (95%CI: 13.5-18.6), p=0.006). Radiological response (ORR, including partial and complete response) was observed in 22/80 (25%) evaluable patients. The proportion of patients with improved/stabilized CPS at 6 months was significantly higher in those achieving ORR: CPS +/=: 14/22 (64%) vs. 21/58 (36%, p=0.027). Of 22 patients with CPS B at BL, 6 (27%) patients died early. At 3 months, CPS improved or stabilized in 7 (32%) patients and deteriorated in 9 (41%) patients. Comparable results were also observed at 6 months (CPS +/=: 7, 32%, -: 5, 23%). In total, 3/22 (14%) patients improved from CPS B to CPS A at both timepoints.

Conclusion: ICI-treatment can induce a prognostically relevant improvement or stabilization of liver function even in patients with Child-Pugh class B at baseline.

Figure: Landmark analysis at 6 months comparing median overall survival (OS) between patients with improved or stable (+/=) as compared to those with deteriorated liver function (-).



P09-19
Comparison between hepatocellular carcinoma in alcohol related disease versus other etiologies - are they the same or not quite yet? A retrospective analysis

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Background and Aims: Hepatocellular carcinoma (HCC) is one of the leading cancers worldwide and typically arises in the setting of advanced chronic liver disease (ACLD), mainly caused by viral hepatitis, alcohol-related liver disease (ARLD) or nonalcoholic fatty liver. In the setting of alcohol consumption an established ACLD is usually required for the development of liver cancer and HCC in alcohol-related liver disease (HCC ARLD) accounts to up 1/3 of global incidence of HCC, although with large variations between regions. We aim to analyze clinical presentation, management and overall survival of HCC in ARLD (HCC-ARLD) versus non-alcohol related disease (HCC NARLD).

Method: Retrospective single-center study, including all patients with HCC diagnosed between 2019 and April 2022 who have been referred to our center. We analyzed patients' demographics, presence of cirrhosis, etiology, presentation (screening, decompensation, constitutional symptoms versus incidental finding), Barcelona Clinic Liver Cancer staging system (BCLC) at diagnosis, Child Pugh, treatment management and overall survival.
T test, Chi-square test, Kaplan Meyer curves and Log Rank test were applied.

Results: 94 patients were included. 54 (57.4%) had ARLD. Mean follow up period was 12.5 months. 28 patients died during this period. Difference in gender was observed, with 53 males in the group of HCC-ARLD versus 30 in HCC NARC ($p<0.01$). No age difference was observed. The majority of patients was diagnosed during screening, but a trend in the cirrhosis decompensation group with 12 patients versus 4 patients in HCC-ARLD and HCC-NARC, respectively, was seen. No differences in BCLC, Child Pugh or overall survival was demonstrated between groups (table 1).

Conclusion: In our study, only gender showed clinical statistical difference between HCC-ARLD versus HCC- NARC. No major differences in clinical presentation, BCLC, Child Pugh and survival was observed. Improvement in detection of HCC-ARLD and screening program of HCC should be standard practice to an early diagnosis and upgrading clinical care in this setting of patients.

Table 1: Comparison between hepatocellular carcinoma patients' demographics, clinical presentation and survival in alcohol related disease versus non-alcohol related disease.

		HCC in ARLD (n=54)	HCC in NARLD (n=40)	*p* value
Gender		53 males	30 males	0.01
Age (in years)		65.06	65.54	0.123
Clinical presentation				0.422
	Screening	30	24	
	Cirrhosis decompensation	12	4	
	Incidental finding	5	6	
	Constitutional symptoms	7	6	
BCLC				0.932
	A	26	19	
	B	8	5	
	C	12	11	
	D	8	5	
Child Pugh				0.833
	A	37	29	
	B	11	8	
	C	6	3	
Mean follow up period (in months)		11.75	13.47	0.171

*ARLC- alcohol-related liver disease; BCLC- Barcelona Clinic Liver Cancer staging system; HCC- Hepatocellular carcinoma; NARLD- non-alcohol related disease

P09-20
PNPLA3 rs738409 C>G polymorphism impact on HCV-Related HCC development in a Brazilian population: preliminary results

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Background and Aims: The PNPLA3 rs738409 C>G polymorphism has been associated with hepatocellular carcinoma (HCC) and liver cirrhosis regardless of the etiology, although the association was stronger with non-viral etiologies. However, the influence of PNPLA3 polymorphism on Hepatitis C Virus (HCV) and whether this polymorphism could be a risk factor for HCV-related HCC is not well defined. Our aim was to evaluate the influence of the PNPLA3 rs738409 C>G polymorphism on the risk of HCC occurrence in HCV patients in Brazil.

Method: This study included 90 patients with HCV-related HCC that underwent liver transplantation or resection at a tertiary center in Brazil and 111 patients non-HCC with HCV, as the control group. The rs738409 polymorphism was detected in the DNA extracted from patients' blood samples using the TaqMan assay. All clinical data were collected using the Research Electronic Data Capture (REDCap) tool. The statistical analyses were performed using Jamovi software version 2.3.23.

Results: In the HCV+HCC group there was a higher proportion of male gender (79.1% vs 45.9%, p<0.001), history of alcoholism (80.5% vs 22.5%, p<0.001) and smoking (68.9% vs 25.2%, p<0.001), however there was no statistical difference in age (p=0.519) and BMI (p=0.403) between both groups. The genotype frequencies of the rs738409 polymorphism in the HCV+HCC group was CC 41,2% CC and CG/GG 58,8% vs controls CC 49,5% and CG/GG 50,5%. The presence of the G allele was not an independent factor associated with the risk of HCC occurrence (r=0,199, p=0.53).

Conclusion: Even in an admixed population such as the Brazilian, there was no association between the PNPLA3 rs738409 C>G polymorphism and the risk of developing HCV-related HCC, as previously shown in published studies in caucasian and oriental population.

P10-01
CT texture analysis may predict genetic profile of mass-forming intrahepatic cholangiocarcinoma

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Background and Aims: Intrahepatic cholangiocarcinoma (ICC) is an aggressive disease with increasing incidence. Comprehensive molecular profiling has shown genetic alterations that are or could be target of systemic therapies. Texture analysis of imaging modalities has led to a reliable prediction of pathology data. The present study aims to elucidate if radiomics extracted from computed tomography (CT) may non-invasively predict ICC genetic alterations.

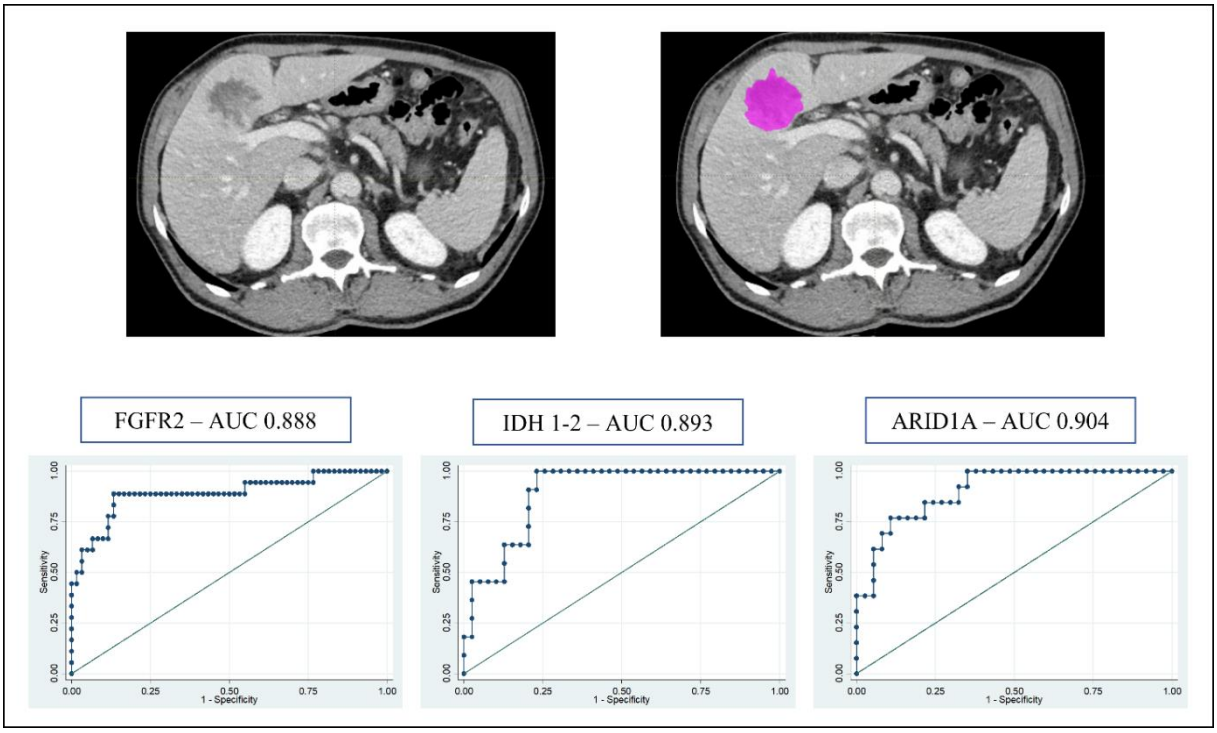
Method: All consecutive patients eligible for systemic therapy for a mass-forming ICC at the authors' institution between January 2016 and June 2022 were considered. Patients were included if they had a complete molecular profiling by NGS. In addition, patients in the early years of the series with a FISH evaluation of *FGFR2* gene fusion/rearrangement were included as well. Genetic analyses were performed on either surgical specimen or biopsy. Additional inclusion criteria were: availability of contrast-enhanced CT at diagnosis before any treatment; adequate quality of the portal phase of the CT for textural analyses. The tumor was manually segmented and radiomic features were automatically extracted using the LifeX software. Multivariate predictive models of the commonest genetic alterations were built exclusively considering the radiomic data.

Results: Overall, 78 patients were enrolled (50 NGS – 28 FISH): 42 (54%) were female, and the median age was 65 (range 33-81) years. The genes with the highest frequency of alteration were *FGFR2* (22%, 17/78, including 8 patients with rearrangement at FISH), *IDH1-2* (22%, 11/50, alteration of *IDH1* in 9 cases), and *ARID1A* 26% (13/50).
The predictive model for *FGFR2* alteration retained seven significant textural features: Volume_voxel (odds ratio, OR=1.001, p=0.031); HUmean (OR=1.309, p=0.005); HU_standard_deviation (OR=1.371, p=0.005); Skewness (OR=3.831, p=0.008); GLCM_Correlation (OR=2.94e-7, p=0.002); GLZLM_SZE (OR=1.31e-10, p=0.004); and GLRLM_SRHGE (OR=0.987, p=0.015). The overall performance was excellent (AUC=0.888). The predictive model for *IDH1-2* alterations included two significant variables (HU_standard_deviation, p=0.018; GLZLM_LZE, p=0.013) and had an AUC=0.893. The predictive model for *ARID1A* included five significant radiomic features (GLCM_Homogeneitiy, p=0.010; GLCM_Correlation, p=0.015; NGLDM_Coarseness, p=0.016; GLRLM_SRLGE, p=0.005; GLRLM_SRHGE, p=0.007) and had an AUC=0.904.

Conclusion: The radiomic features extracted from CT at diagnosis of ICC may provide a reliable non-invasive prediction of its genetic status. Our preliminary data need prospective validation on larger series

but could open new perspectives in the non-invasive assessment of ICC molecular profiling, with a major impact on therapeutic strategies.

Figure:



P10-03-YI
Sequential therapies after atezolizumab plus bevacizumab or lenvatinib first-line treatments

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Background and Aims: Today the most used first-line treatments in hepatocellular carcinoma (HCC) patients are lenvatinib (L) and atezolizumab plus bevacizumab (AB). All the data available about second-line therapies derive from trials conducted in patients who progressed to first-line sorafenib (S) therapy. The aim of this retrospective proof-of-concept study is to compare different second-line drugs for HCC patients progressed to first-line L or AB.

Method: The overall cohort included 2225 consecutive patients from 5 countries (Italy, Germany, Portugal, Japan, and Republic of Korea). A total of 1381 patients had progressed disease (PD) at first-line therapy. 917 patients were in L first-line arm, and 464 patients were in AB first-line arm.

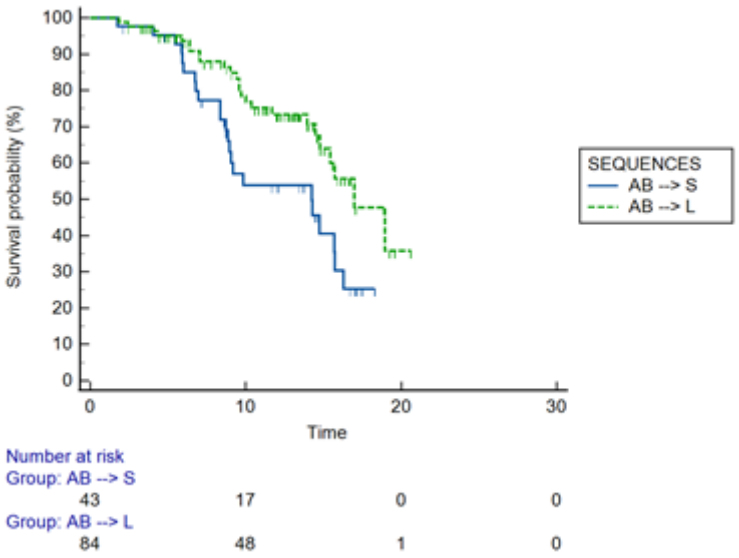
Results: 49.6% of PD patients received second-line therapy without any statistical difference in OS between L first-line arm (20.6 months) and AB first-line arm [15.7 months; p = 0.12; hazard ratio (HR) = 0.80].

In L first-line arm, there was a statistical difference between second-line therapy subgroups [p = 0.04; S HR = 1; trans-arterial chemo-embolization (TACE) HR = 0.65; immunotherapy (I) HR = 0.69; other therapies (O) HR = 0.85]. 40.1% of patients were treated with S achieving an OS of 15.8 months; 36.8% of patients underwent TACE with an OS of 24.7 months; 13.1% of patients were treated with I not reaching a median OS; 10.0% of patients received O with an OS of 20.8 months. Patients who underwent TACE had a significative longer OS than patients who received S (p < 0.01; HR = 0.64).
In AB first-line arm, there was a statistical difference between second-line therapy subgroups [p < 0.01; S HR = 1; L HR = 0.50; cabozantinib (C) HR = 1.34; TACE HR = 0.39; O HR = 0.54]. 18.4% of patients were treated with S achieving an OS of 14.2 months; 36.0% of patients were treated with L with an OS of 17.0 months; 9.9% of patients received C achieving an OS of 12.4 months; 11.6% of patients underwent TACE with an OS of 15.9 months; 24.0% of patients received O not reaching a median OS. Patients who received L had a significative longer OS than patients treated with S (p = 0.01; HR = 0.45). Patients who underwent TACE had a significative longer OS than patients who received S (p < 0.05; HR = 0.46).

No other statistical differences were highlighted between the other subgroups and patients treated with S in both first-line arms.

Conclusion: The L – I and AB – L sequences are able to achieve the longest median survivals. For patients eligible for locoregional therapy after first-line systemic therapy, TACE has been shown to achieve longer survivals than second-line S in both first-line arms.

Figure:



P10-08-YI
Collagen proportionate area (CPA) measurement of liver parenchyma and hepatocellular carcinoma (HCC) can predict HCC recurrence after liver resection

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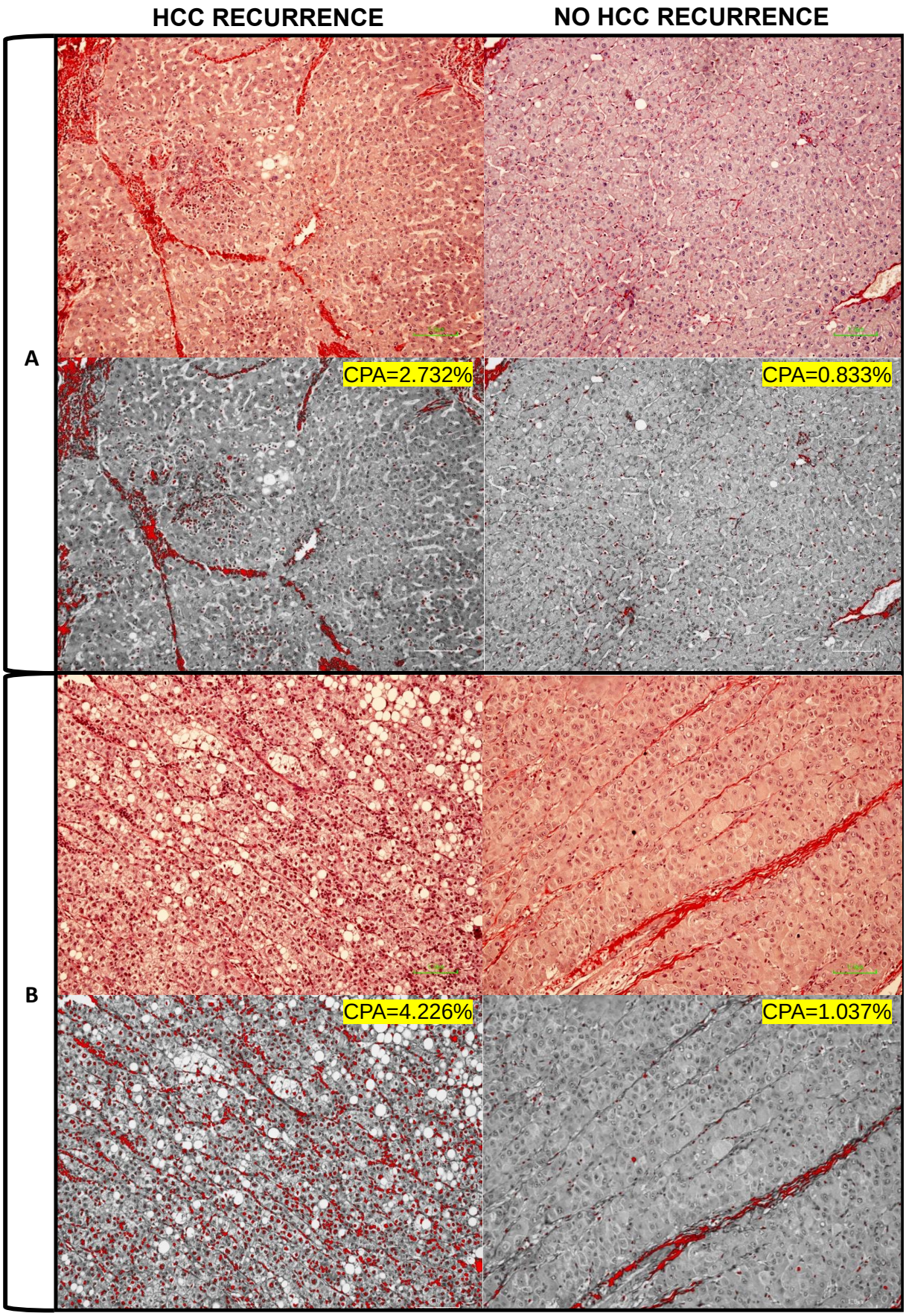
Background and Aims: Hepatocellular carcinoma (HCC) is a frequent complication in patients with advanced chronic liver disease (ACLD) with an HCC recurrence (HCC-r) rate of around 70% after liver resection (LR) at five years. Previous studies have found several predictive variables for HCC-r mainly related to the HCC characteristics or related to the stage of liver disease and the degree of portal hypertension. Collagen proportionate area (CPA) measurement is a quantitative automated method for estimating fibrous tissue in liver biopsies by determining the collagen deposition percentage of the total biopsy area. Thus, we aimed to evaluate the predictive role of CPA in HCC-r after curative LR.

Method: From a cohort of 175 patients with primary HCC eligible for LR prospectively enrolled and followed for at least 30 months or until HCC-r, we analysed the biopsy specimen sampled obtained during LR surgery of 54 patients with a 3:2 ratio of HCC-r. For each patient, ten images of liver sections stained with Sirius red for collagen fibres were acquired from the non-neoplastic liver parenchyma adjacent to the tumour (liver-CPA; **Fig1A**) and HCC tissue (HCC-CPA; **Fig1B**) with an Axiocam 305 colours mounted on a Zeiss Axioscope AX10 microscope. CPA was measured by free public-domain software developed by the National Institutes of Health (NIH-ImageJ). For analyses, the median CPA values were evaluated. Logistic regression analyses were performed to evaluate the prediction of HCC-r.

Results: 35 out of 54 (65%) patients developed HCC-r, 25 (46.3%) patients developed an early (≤24 months) HCC-r, and 10 (18.5%) patients developed late (>24 months) HCC-r. Patients were mainly males (88.9%), with a median age at HCC diagnosis of 73 [68 - 85] years; the prevalent etiology was viral (81.5%). 47 (87%) median MELD score was 8 (7 - 9). 98.15% was Child-Pugh A with median alpha-fetoprotein values of 6 (3 - 38) ng/ml. Liver and HCC-CPA were significantly higher in patients with HCC-r than those without HCC-r (Fig.1). Liver-CPA significantly correlates with LSM (R= 0.703; p= 0.05). Among all variables evaluated, HCC-r was significantly predicted by liver-CPA (OR 2.672 [95%CI 1.142 - 6.255] p= 0.002) and HCC-CPA (OR 1.0002 [95%CI 1.000 - 1.0004]; p= 0.021), number of HCC nodules (OR 2.489 [95%CI 1.876 - 7.071]; p= 0.054), microvascular invasion (p= 0.036), and macrovascular invasion (p= 0.048).

Conclusion: CPA is a good quantitative and automated method for estimating fibrosis tissue, and together with already known predictive factors (such as histological differentiation and extent of invasion, satellite nodules, size and the number of nodules, Metavir score, LSM and SSM) can predict the recurrence of HCC after liver resection. Further studies should confirm our results to recommend adding CPA to the standard pathological analysis of liver resected HCC patients.

Figure:


HCC RECURRENCE
NO HCC RECURRENCE
A
CPA=2.732%
CPA=0.833%
B
CPA=4.226%
CPA=1.037%

P10-10
Adverse events related to atezolizumab-bevacizumab is comparable among CTP-A and B and more safer than TKIs for Hepatocellular carcinoma

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Background and Aims: Atezolizumab-bevacizumab (atezo-bev) is the recommended first-line therapy for patients with unresectable hepatocellular carcinoma (uHCC). Adverse events (AEs) are common with atezo-bev therapy and variceal bleeding is the most feared complication.

Method: Electronic databases were searched for relevant articles on atezo-bev therapy. We aimed to assess the cumulative incidence of AEs with atezo-bev therapy and compare the same among CTP-A and B and also compare the incidence of AEs with tyrosine kinase inhibitors (TKIs).

Results:47 studies (5400 patients) were included for analysis. The cumulative incidence of AEs (any grade) was 82·7% (77·8–86·7; 2619/3332;I^2=89·25). The cumulative incidence of grade ≥3 AEs was 31·8% (25·6–38·7; I^2=93·56) among 29 studies and 3855 patients. The proportion of patients discontinuing atezo-bev was 13·8% (10–18·7; I^2=88·73) among 2651 patients. The incidence of grade ≥3 events among CTP A and CTP B was comparable among four studies reported (OR,0·89 [0·45–1·74];P=0·74). The cumulative incidence of variceal bleeding was 4·7% (103/2062). (Table) 79% (1130/1429) of patients underwent screening endoscopy prior to atezo-bev therapy. Of them, only 46% of patients had varices. Furthermore, 54% (250/465) of patients had received treatment for varices either in the form of ligation and/or non-selective beta-blocker. The incidence of variceal bleeding was similar between CTP A (8·55%; 29/342) and B (9%; 10/111) (OR, 0·83 [0·2–3·41];P=0·8;I^2=56·98) among three studies. The incidence of variceal bleeding was significantly more in patients with portal vein thrombosis (PVT) (11·6%;24/208) than those without PVT (6·2%;21/376) (OR, 2·05 [1·1–3·82];P=0·02). The incidence of variceal bleeding was similar among those, who received treatment prior to therapy with ligation and/or NSBB (8·2%;7/85) and those who did not receive (4·1%; 10/241) (OR,2·12[0·63-7·17];P=0·22). The cumulative incidence of AEs was similar among those who received atezo-bev and TKI (OR, 0·69 [0·14–3·5];P=0·53; I^2=86·46). The cumulative incidence of grade ≥3 AEs was also comparable among those treated with atezo-bev and TKIs among six studies (OR,0·86 [0·61–1·2];P=0·38;I^2=89·1). The incidence of grade 3 events of hypertension (OR,1·01 [0·64–1·6];P=0·95) and gastrointestinal bleeding (OR, 1·55 [0·64–3·75];P=0·32) was similar among those treated with atezo-bev and TKIs. The incidence of anorexia (OR, 0·28 [0·17–0·44];P<0·001), fatigue (OR,0·43 [0·28–0·68];P<0·001), diarrhea (OR, 0·3[0·16–0·56];P<0·001), hand-foot skin reaction (OR, 0·19 [0·08–0·44];P<0·001) and proteinuria (OR, 0·76 [0·56–1·03;P=0·08) were significantly lower with atezo-bev than with TKIs especially lenvatinib.

Conclusion: AEs are comparable among CTP-A and B in patients treated with atezo-bev. Atezo-bev is safer than TKIs. PVT is a determinant of variceal bleeding.

Figure:

Adverse events among patients treated with atezo-bev

Adverse events	n/N	Incidence
Hypertension		
Cumulative	1082/4073	27% (23·3-31·2); I²=84·42
Grade ≥3	244/3804	6% (4·7-7·8); I²=64·87
Fatigue		
Cumulative	987/4060	25·1% (22·1-28·4); I²=76·71
Grade ≥3	58/3753	2% (1·6-2·6); I²=0·0
Anorexia		
Cumulative	623/3240	18·8% (15·8-22·2); I²=76·36
Grade ≥3	46/2978	2% (1·4-2·4);I²=0
Fever		
Cumulative	235/1434	16·3% (12·4-21); I²=75·22
Grade ≥3	19/1344	1·8% (1·2-2·7); I²=0
Proteinuria		
Cumulative	985/3822	25·2% (22·2-28·6); I²=75·55
Grade ≥3	207/3445	6·3% (5·1-7·8); I²=42·84

Adverse events	n/N	Incidence
Diarrhea		
Cumulative	281/2791	9·3% (7·3-11·8); I²=69·57
Grade ≥3	26/2759	1·3% (0·1-1·9); I²=0·0
Gastrointestinal		
bleeding	128/2255	5·6% (4-8); I²=70·87
Variceal bleeding	103/2062	4·7% (3·3-6·7); I²= 63·68
Hypothyroidism		
Cumulative	178/2591	7% (5·2-9·4); I²=64·1
Grade ≥3	4/2284	0·5 (0·3-1); I²=0·0
AST elevation		
Cumulative	407/1547	25% (18·1-33·2); I²=90
Grade ≥3	83/1497	6·4% (5·1-8); I²=9·25
Others (cumulative)		
Thrombocytopenia	228/1145	15·3% (8·4-26); I²=93·3
Infusion reaction	47/762	3·4% (1·4-7·7); I²=69·95
HFSR	37/1545	3·1% (1·5-6); I²=67·21
iRAE	181/1537	10·2% (6·9-14·8); I²=72·53

P10-11
Alpha-Fetoprotein Response Patterns after Y-90 Radioembolization for Intermediate-to-Advanced Hepatocellular Carcinoma Predict Disease Progression and Survival

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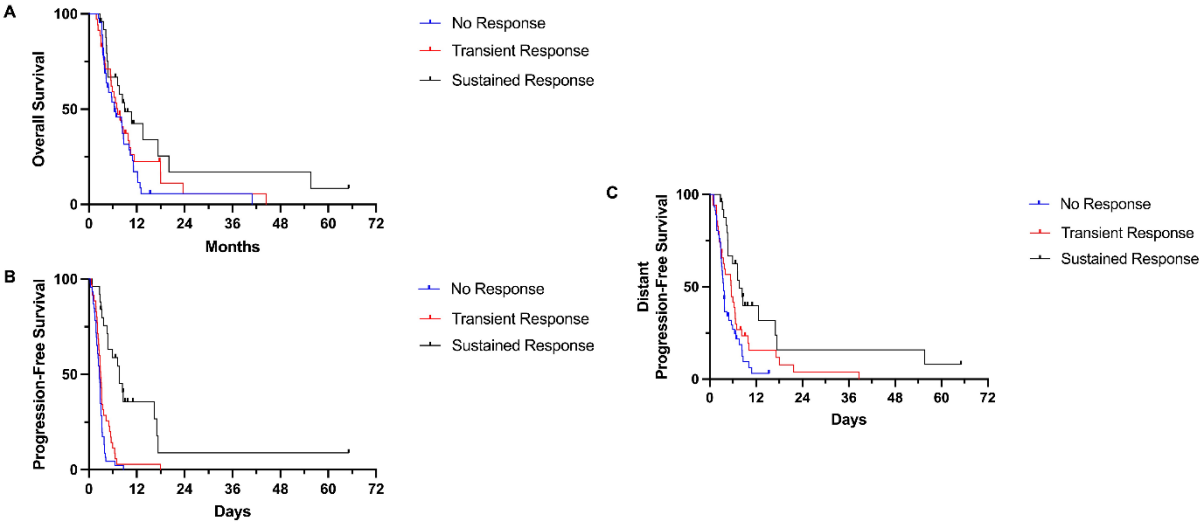
Background and Aims: Alpha-fetoprotein (AFP) response patterns after Yttrium-90 (Y-90) radioembolization are not well understood and not formally part of post-treatment surveillance guidelines. The aim of our study was to describe AFP response patterns in patients with AFP-producing BCLC stage B or C hepatocellular carcinoma (HCC) after Y-90 radioembolization and their correlation with overall survival (OS), distant progression-free survival (DPFS), and progression-free survival (PFS).

Method: This single-center, retrospective study evaluated 106 patients from 2011-2022 with AFP-producing HCC (≥20 ng/mL) without extrahepatic metastases who underwent Y-90 radioembolization. Pre-treatment and all post-treatment imaging were evaluated. Modified Response Evaluation Criteria in Solid Tumors (mRECIST) was applied. AFP response was categorized into 3 patterns: 1) no response (<20% AFP decrease or increase), 2) transient response (decrease by ≥20%, followed by increase at any time point thereafter), and 3) sustained response (persistent AFP decrease by ≥20%). OS, DPFS, and PFS were estimated using Kaplan-Meier curve analyses. Multivariate analyses for OS and PFS were assessed with Cox proportional hazards regression.

Results: Twenty-five (24%) patients had sustained response, 35 (33%) had transient response, and 46 (42%) had no response. Median OS of sustained, transient, and no response were 9.0, 7.3, and 6.5 months, respectively (p=0.019). Median DPFS of sustained, transient, and no response were 7.7 months, 5.5 months and 3.6 months, respectively (p<0.001). Median PFS of sustained, transient, and no response were 7.7, 2.9, and 2.7 months, respectively (p<0.001; summarized in the Figure). Eastern Cooperative Oncology Group Performance Scale, AFP response, and Child-Pugh class were independent prognostic indices of OS. Baseline AFP and AFP response were independent prognostic indices of PFS.

Conclusion: Among patients with advanced HCC after Y-90 radioembolization, no AFP response is associated with early progression or death and transient response is associated with delayed progression or death. More than half of patients who have an initial AFP response relapse and have only slightly better outcomes compared to those with no response.

Figure: Kaplan-Meier analysis of (A) overall survival, (B) distant progression-free survival, and (C) progression-free survival stratified by AFP response pattern. Each vertical line on the curve represents a censored event.



P10-15
Long-term survival in patients with unresectable liver cancer treated with lenvatinib

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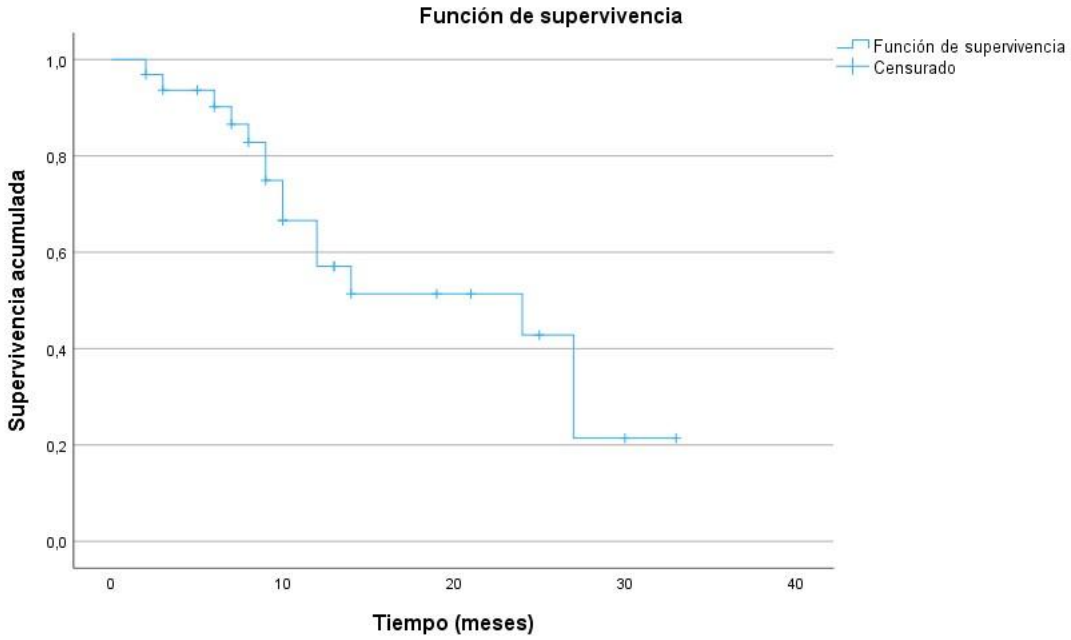
Background and Aims: Lenvatinib was approved in our centre in March 2020 for unresectable liver cancer in first-line. Nowadays there are few real-world studies in European patients treated with lenvatinib who differ from Asian populations in demographics and etiologies. The aim of this study is to assess the results of this therapy in our patients, determining overall survival (OS) and time to progression (TTP).

Method: This is a descriptive and retrospective study of all the patients treated with lenvatinib, according to REFLECT criteria, in our centre from March 2022 to December 2022.
Data was analysed with SPSS IBM version 29.

Results: 32 patients were treated with lenvatinib. The mean age at diagnosis was 67.7 years old. 25 patients were men (78%). All of them were Caucasians. 26 were cirrhotics (81.3%), 11 had oesophagic varices (34.4%) and all of them were Child-Pugh A. Hepatitis C was involved in 15 (46,9%), alcohol in 14 (43.8%), hepatitis B in 4 (12,6%) and MAFLD in 2 (6,3 %). 10 (31.3%) were BCLC-B and 22 (68.8%) C at the beginning. 13 patients (40.6%) had not received any treatment before lenvatinib. 10 patients (31.3%) had undergone chemoembolization. There have been 15 deaths during the follow up (33 months). Our median OS was 24 months (CI 5% 4.6-43.4).
All of our patients had adverse events (AE). Only 3 (9.4%) had to discontinue the drug. The most frequent AE have been asthenia in 23 (72%), weight loss in 20 (62.5%), high blood pressure in 20 (62.5%), hypothyroidism in 14 (43.8%) and diarrhoea in 13 (40.6%). 4 presented proteinuria (12.5%). 12 (37,5%) are still in treatment (to 19th December 2022). Treatment was discontinued due to tumour progression in 7 (21,9%) cases, performance impairment in 5 (15.6%), proteinuria in 1 (3.1%) and gastrointestinal bleeding in another one (3.1%).
8 patients (25%) received a second-line therapy. We could evaluate radiological response in 21 cases (65.6%): no one presented complete response, 3 (14.3%) had partial response, 7 (33.3%) maintained stable disease and 11 (52.5%) progressive disease. Median TTP was 7 months (95% CI 2.5-11.4). After progression, patients were switched to a second line.
We studied variables of sex, etiology, liver function, tumour characteristics and the presence of adverse events. We did not find any significative association with better survival, but a negative association with asthenia.
The limitations of our study are the number of patients and its retrospective character.

ConclusionWe reached an OS of 24 months, which is higher to the reported to date. Nevertheless, our TTP was no different. Patients with lenvatinib alone could reach a similar OS to the achieved with atezolizumab plus bevacizumab. A strict selection of patients, the different etiologies distribution of our patients and the use of a second-line treatment in 25% of them, may explain this prolonged survival compared with that published before.

Figure



Kaplan-Meier analysis.

P10-16
Downstaging hepatocellular carcinoma to transplant criteria using lenvatinib

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Background and Aims: Systemic therapy for hepatocellular carcinoma class BCLC – B/BCLC-C with vascular invasion, has shown response rates upto 40%. Liver transplant is the only curative modality for these patients, but there is scarce data to support the same. Here we report our experience with downstaging HCC using Lenvatinib, a tyrosine kinase inhibitor.

Method: Retrospective data from 2020-2022 of 25 patients with advanced HCC (BCLC – B out of seven, BCLC – C) were given Lenvatinib with/without locoregional therapy.

Results: 16/25 patients followed up for atleast 3 months. 8 patients were BCLC-B and 17 patients were BCLC-C (VP1 – 1; VP2 – 5; VP3 – 4; VP4 – 7). 5/16 patients were downstaged to within UCSF for atleast 3 months (2 VP3). 3/5 patients (BCLC-B) underwent living donor liver transplantation. Criteria for transplant were within UCSF, AFP <100 mg/ml, PET negative. Lenvatinib was stopped 2 weeks prior to the transplant. There were no peri-operative complications like bleeding, delayed wound healing, infections. Biliary complications, rejection were not noted. Patients are recurrence free at 2 years, 1 year and 3 months post transplant. Explant showed positivity for beta catenin.

Conclusion: Patients downstaged to within UCSF criteria using Lenvatinib may undergo liver transplantation without increased peri-operative complications or recurrence. There is need to identify molecular markers for predicting response to Lenvatinib.

Figure:

Sr.No.	BCLC stage	PVTT	Treatment for HCC	FU – 3 month Response to the treatment	Outcome
1	C	Mets	Lenvatinib	LTFU	LTFU
2	C	vp3	Lenvatinib+TACE	Complete response	Continue Lenvatinib – Workup for transplant
3	C	vp4	Lenvatinib+TARE	LTFU	LTFU
4	C	Mets	Lenvatinib	LTFU	LTFU
5	C	vp4	TARE+Lenvatinib	LTFU	LTFU
6	C	vp4	Lenvatinib	Stable	Continue Lenvatinib
7	C	vp4	Lenvatinib	Partial Response	Continue Lenvatinib
8	B	-	TACE+Lenvatinib	Complete response	Transplanted
9	B	-	Lenvatinib	Complete response	Continue Lenvatinib – Workup for transplant
10	C	vp3	Lenvatinib	Intolerance	stopped
11	C	vp3	Lenvatinib	Progression of disease	stopped
12	B	-	Lenvatinib	Complete response	Transplanted
13	B	-	Lenvatinib	LTFU	LTFU
14	C	vp1	Lenvatinib	LTFU	LTFU
15	C	vp4	Lenvatinib	Partial	Dose reduced
16	C	-	Lenvatinib	LTFU	LTFU
17	B	-	Lenvatinib	LTFU	LTFU
18	C	vp4	Lenvatinib	Intolerance	stopped
19	B	-	TACE+Lenvatinib	Complete response	Transplanted
20	C	vp3	Lenvatinib	Partial response	Continue Lenvatinib
21	B	-	Lenvatinib	Partial response	TACE done on 8 Sep 2021
22	C	vp3	Lenvatinib	Intolerance	stopped
23	C	vp4	Lenvatinib	Progression of disease	Expired
24	B	-	Lenvatinib	Stable	Continue Lenvatinib
25	B	-	Lenvatinib	Stable	Stopped due to worsening liver functions

BCLC – Barcelona Clinic for Liver Cancer
TACE – Trans Arterial Chemo Embolisation
TARE - Trans Arterial Radio Embolisation
LTFU – Lost to follow up

P10-17
Peritumoral portal enhancement during transarterial chemoembolization – a potential prognostic factor for patients with hepatocellular carcinoma

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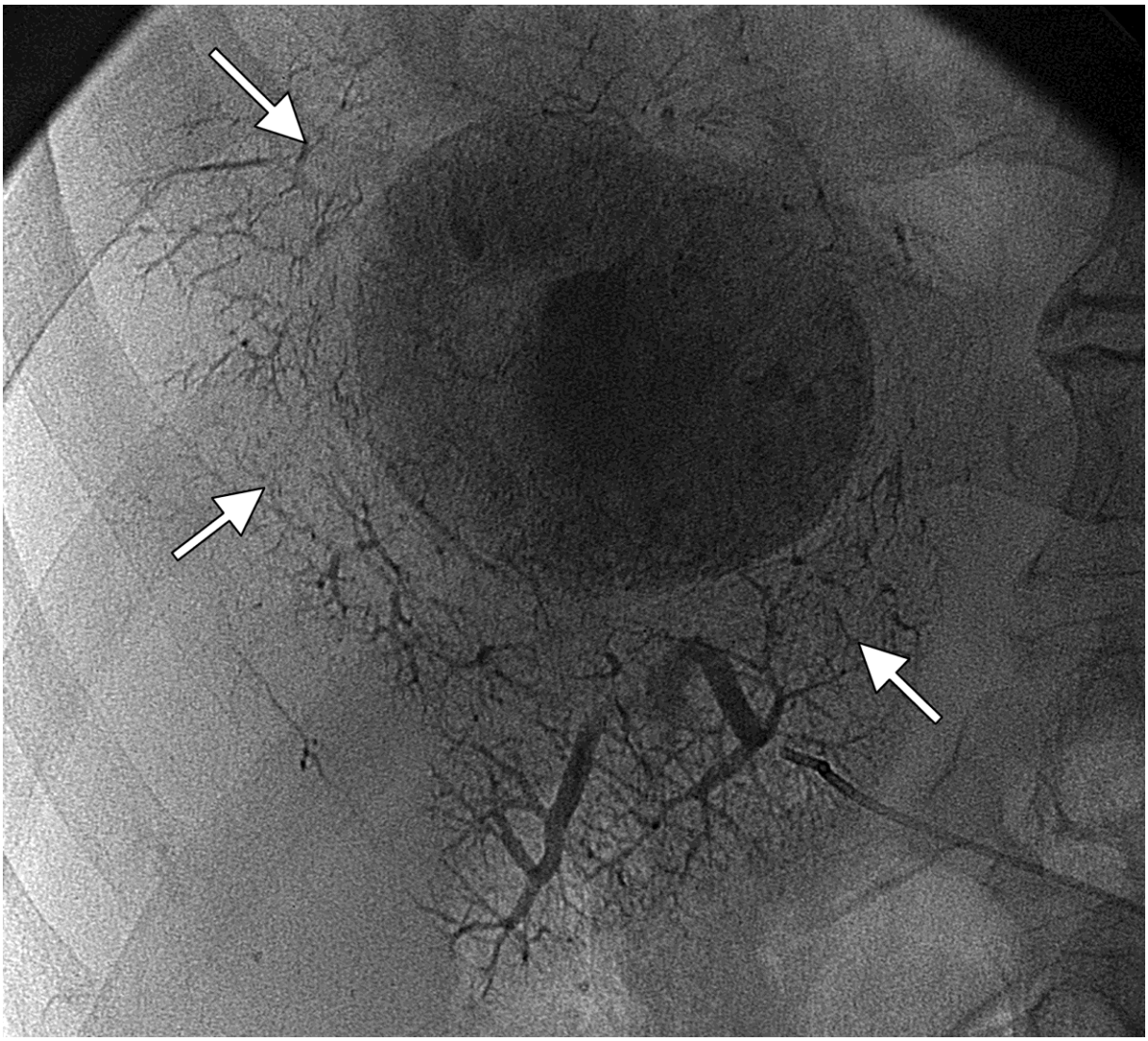
Background and Aims: Tumor response and survival varies in patients treated with transarterial chemoembolization (TACE) for intermediate stage hepatocellular carcinoma (HCC) and may be associated with several factors. The aim of this study was to evaluate safety and efficacy of TACE in patients with intermediate stage HCC and to identify factors related to tumor response and survival.

Method: Consecutive patients with HCC treated with TACE between September 2008 and September 2018 were retrospectively reviewed.

Results: In 87 patients (mean age 68 ± 9 years, 71 men), 327 TACE treatments were performed (mean 3/patient, range 1-12). Mean and median overall survival was 32 and 19 months respectively. Survival rates at 30 days, 1, 3 and 5 years were 99 %, 71 %, 19%, and 8 %, respectively. Objective response (OR) was seen in 84 % and disease control (DC) was seen in 92 % of the patients. Patients in whom peritumoral portal lipiodol enhancement (PPLE) was seen during TACE had better OR (97 vs 73%, p = 0.007) and DC (100 vs 85%, p = 0.024), and a reduced risk of death (hazards ratio (HR) 0.52, 95% CI 0.32-0.86) compared to those without PPLE. Severe adverse events were rare (15%) and occurred more often in patients with a larger tumor size.

Conclusion: TACE was effective and safe in patients with intermediate stage HCC. Patients with PPLE during TACE had better tumor response and longer survival than those without PPLE. Severe adverse events occurred more often in patients with larger tumors.

Figure: Radiographic image displaying peritumoral portal lipiodol enhancement (PPLE) (arrows) during transarterial chemoembolization (TACE) for hepatocellular carcinoma (HCC). In this 86-year-old man, pathological anatomical diagnosis had demonstrated a highly differentiated tumor. Complete remission was achieved, and the patient was still alive at follow-up after 8 years.



P10-18
Gender differences in patients with hepatocellular carcinoma

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Background and Aims: Gender disparities in hepatocellular carcinoma (HCC) presentation and prognosis have been noted. These have been attributed to the interplay of behavioural and biological factors that are conditioned, in part, by geographical location and ethnicity. Gender differences, among other factors, influence the HCC surveillance strategy. However, European data on this matter is scarce. The aim of this study was to assess gender differences in Croatian HCC patients treated at our Centre.

Method: This retrospective study included 343 Caucasian patients diagnosed with HCC between 2005 and 2022 (80.4% male). Patients records were retrieved from the hospital's database. The statistical package "R" was used for the analysis, with a significance set at $p<0.05$.

Results: Men were more likely to have a diagnosis of liver cirrhosis established before HCC development (OR 1.14, p=0.66), to be diagnosed by surveillance (OR 1.07, p=0.95) and in the curative stage of HCC (OR 1.12, p=0.7). Women were more likely to be diagnosed due to HCC symptoms (OR 1.8, p=0.05) and with poorly differentiated tumour (OR 3.33, p=0.03). Men were more likely to present with AFP (Alpha fetoprotein) negative HCC (OR 3.65, p=0.0013). Women with early HCC had significantly higher AFP when compared to men (median (w) 95.33, median (m) 8.4, p=0.0014). Men were more likely to have a full liver disease etiology workup (OR 1.25, p=0.42). The most common etiology in men was alcoholic liver disease (54.5% in relation to other causes) while viral hepatitis was the most common in women (46%). There was no difference in overall survival between men and women (p=0.36).

Conclusion: Women were more likely to present with symptomatic and AFP positive tumour and less likely than men to have the diagnosis of cirrhosis established before HCC develops. We propose surveillance strategy to focus on identifying women at high risk of HCC and to incorporate AFP into their monitoring.

P10-20
Assesment of GALAD score as diagnostic and prognostic factor of denovo hepatocellular carcinoma post treatment with direct acting antiviral agents

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Background and Aims: The GALAD score was proposed as a statistical model for estimating the presence of Hepatocellular carcinoma (HCC) in patients with chronic liver disease. It is derived from Gender, Age, Lens culinaris agglutinin reactive fraction of AFP (AFP-L3), alpha-fetoprotein (AFP) and Des-gamma-carboxy-prothrombin (DCP). It was shown to be highly accurate model for the detection of HCC.

Method: This study was performed in two phases and conducted on 176 Egyptian patients, 88 patients developed hepatitis C-related HCC after treatment with DAAs. In addition, 88 patients with liver cirrhosis without HCC and previously treated with DAAs as a control group. To perform GALAD Score, We measured AFP-L3, alpha-fetoprotein (AFP) and DCP. We studied the relation between GALAD score and the development, characteristics , and outcome of DAAs-related HCC

Results: There was no difference between both groups in their age or gender distribution. In the DAAs related-HCC group, the median duration between HCC development and the end of DAAs therapy was 12 months.

Mean GALAD score value was significantly higher among patients with DAAs related-HCC when (4.32 ± 2.47 vs. 1.32 ± 1.46, p value <0.001). GALAD score (Odds ratio 2.378, 95% CI 1.171-4.831, P-value 0.017) and LSM assessed via fibroscan (Odds ratio 1.140, 95% CI 1.007-1.291, P-value 0.038) were the independent predictors for the development of DAAs-related HCC (p=0.017, p=0.038). GALAD score was of low diagnostic accuracy in prediction of mortality among HCC patients (AUC=0.564, 95% CI: 0.434 – 0.693).

We found that baseline GALAD score was significantly higher among patients who experienced HCC recurrence after complete ablation (6.16 ± 2.23 versus 4.18 ± 2.45, P value 0.032). multivariate regression analysis revealed that CHILD score was the only significant predictor of recurrence among patients with HCC (p=0.02)

Conclusion: GALAD score could predict the development of HCC in patients previously treated with DAAs

Figure:

Diagnostic accuracy of GALAD score in early prediction of HCC

Test Result Variable(s)	Area under curve	P value	95% Confidence Interval		Cutoff value	Sensitivity %	Specificity %
			Lower Bound	Upper Bound			
AFP	0.854	<0.001	0.794	0.915	7.9	74.4	94.3
DCP	0.860	<0.001	0.800	0.920	366	82.6	86.4
AFP-LP3	0.796	<0.001	0.730	0.862	8.25	68.2	77.3
GALAD	0.849	<0.001	0.794	0.905	3.805	59.3	97.7

P11-01
Tumour associated endothelial expression of B7-H3 predicts survival in hepatocellular carcinomas

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Background and Aims: B7-H3 (also known as CD276) is a newly found molecule of B7 family, which is an immunomodulatory transmembrane N-linked glycoprotein that is overexpressed in a number of solid tumours. Current studies describe B7-H3 as a T cell inhibitor that promotes tumour aggressiveness and proliferation. Therefore, this study aimed to investigate whether tumour and endothelial H7-H3 expression were associated with tumour behaviour and prognosis in hepatocellular carcinoma (HCC) after surgical resection.

Method: Consecutive patients with HCC who had undergone surgical resection, or liver transplantation were included from two tertiary hospitals, and the training set (n = 626, surgically resected) and the validation set (n = 100, transplanted) were established. Tissue microarray (TMA) was prepared from HCC tissues and adjacent non-tumour tissues and their associations with clinical characteristics, RFS (recur-free survival), and OS (overall survival) were analyzed according to the level of B7-H3 expression.

Results: Among 626 surgically resected training sets (mean age 57.6, 79.6% of male, 93.9% of Child A, 17.3% of multiple tumours, mean tumour size 4.8 cm), 17.8% (110/626) and 55.0% (344/626) showed moderate to strong tumour and endothelial B7-H3 expression in TMA, respectively.
High endothelial B7-H3 expression group (n=344) showed significantly younger age (56.6 vs. 58.6, p=0.014) and a higher proportion of multiple tumors (20.4% vs. 13.5% p=0.023), microvascular invasion (50.0% vs. 32.3%, p<0.001), and ES grade 4 (13.1% vs. 30.6%, p<0.001) than low endothelial B7-H3 expression group (n=282). The high endothelial B7-H3 expression group showed significantly shorter RFS (median 22 mo. vs.47 mo., p<0.001) and OS (median 127 mo. vs. NR, p<0.001) than the low endothelial B7-H3 expression group. The high tumour B7-H3 expression group showed significantly poorer OS (median 115 mo. vs.139 mo., p<0.026) than the low tumour B7-H3 expression group, however, RFS was not (median 21 mo. vs.35 mo., p=0.085). In the Multivariate Cox regression analysis, high endothelial B7-H3 expression is independently associated with poor prognosis (HR 1.29, 95% CI 1.04-1.59, p=0.019) along with tumor size (HR 1.07), microvascular invasion (HR 1.31), multiple tumors (HR 1.59), and Child-Pugh score (HR 1.17), however high tumor B7-H3 was not.
Among 100 transplanted validation set (mean age 56.9, 84% of male, 37% and 35% of Child B and C, 79% of multiple tumours, mean tumour size 4.1 cm), the increased endothelial B7-H3 expression was a significant prognostic factor for poor OS (p=0.029 for trend).

Conclusion: The endothelial high expression of B7-H3 is associated with aggressive tumour characteristics and showed poor PFS and OS. Further studies are needed on the efficacy of adjuvant immunotherapy in HCC patients with high B7-H3 expression.

P11-03-YI
Incidence and risk factors of esophagogastric varices bleeding in patients with advanced Hepatocellular Carcinoma treated with Lenvatinib

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Background and Aims: Lenvatinib (LEN) is among the drugs used in the forefront treatment of patients with advanced hepatocellular carcinoma (HCC) and in the future it could be part of therapeutic combinations with immunotherapy. However, the presence of esophagogastric varices (EGV) and the risk of bleeding might either contraindicate or limit this therapeutic choice. Thus study aimed to assess prevalence, risk factors and clinical consequences of EGV in LEN-treated patients with HCC.

Method: Among 816 patients of a large international cohort of patients treated with LEN for HCC not eligible for other therapies, we selected those with an upper-gastrointestinal endoscopy (UGE) available in the 6 months before treatment start. Primary end-points were: prevalence and risk factors for EGV bleeding during LEN treatment; secondary end-points were prevalence and risk factors for presence of high risk EGV at baseline.

Results: We enrolled 535 patients with baseline UGE [median age 72 years, 78% male, 63% viral aetiology, 89% Child-Pugh A, 16% neoplastic portal vein thrombosis (nPVT), 56% BCLC-C]. At baseline, 301 (56%) patients were EGV free. Among the 234 patients with EGV (44%), 206 had esophageal varices (EV), 16 gastric varices (GV) and 12 both; 70/234 (30%) were high-risk EGV (small EV with red signs, medium/large EV, any GV) at baseline and 59 of them were treated with primary prophylaxis (non-selective beta-blockers 25, endoscopic band ligation 32 and both 2). Child-Pugh B (OR 2.11; 95% CI 1.18-3.77, p = 0.01), platelets < 150,000 (OR 3.19; 95% CI 2.17-4.70, p < 0.001) and nPVT (OR 2.44; 95% CI 1.48-4.02, p < 0.001) independently predicted presence of EGV; while Child-Pugh B (OR 2.12; 95% CI 1.08-4.17, p=0.03), platelets < 150,000 (OR 2.47; 95% CI 1.35-4.50, p = 0.003) and nPVT (OR 2.54; 95% CI 1.40-4.61, p = 0.002) independently predicted high risk EGV. During LEN therapy, 17 patients bled from EGV (3 grade 2, 11 grade 3-4 and 3 grade 5): prevalence of EGV bleeding was 3% overall, 7% among patients with EGV and 17% among those with high-risk varices. Among the 234 patients with baseline EGV, the only independent predictor of bleeding was the presence of high-risk varices (HR 6.94; 95% CI 2.23-21.57, p = 0.001). Risk of EGV bleeding can be stratified according to Child-Pugh B, presence of nPVT and platelets < 150.000/uL into low (0/3 risk factors, 6-months cumulative incidence 0.77%), intermediate (1/3 risk factors, 6-months cumulative incidence 2.31%) and high (2/3 or 3/3 risk factors, 6-months cumulative incidence 7.40%).

Conclusion: In HCC patients treated with lenvatinib, the risk of EGV bleeding is low but it increases in patients with high-risk EGV at baseline. A risk stratification for high-risk EGV and bleeding can be applied for decision-making, according to liver reserve, platelet count and nPVT.

P11-04-YI
Time-trends in cholangiocarcinoma incidence - a Danish nationwide cohort study

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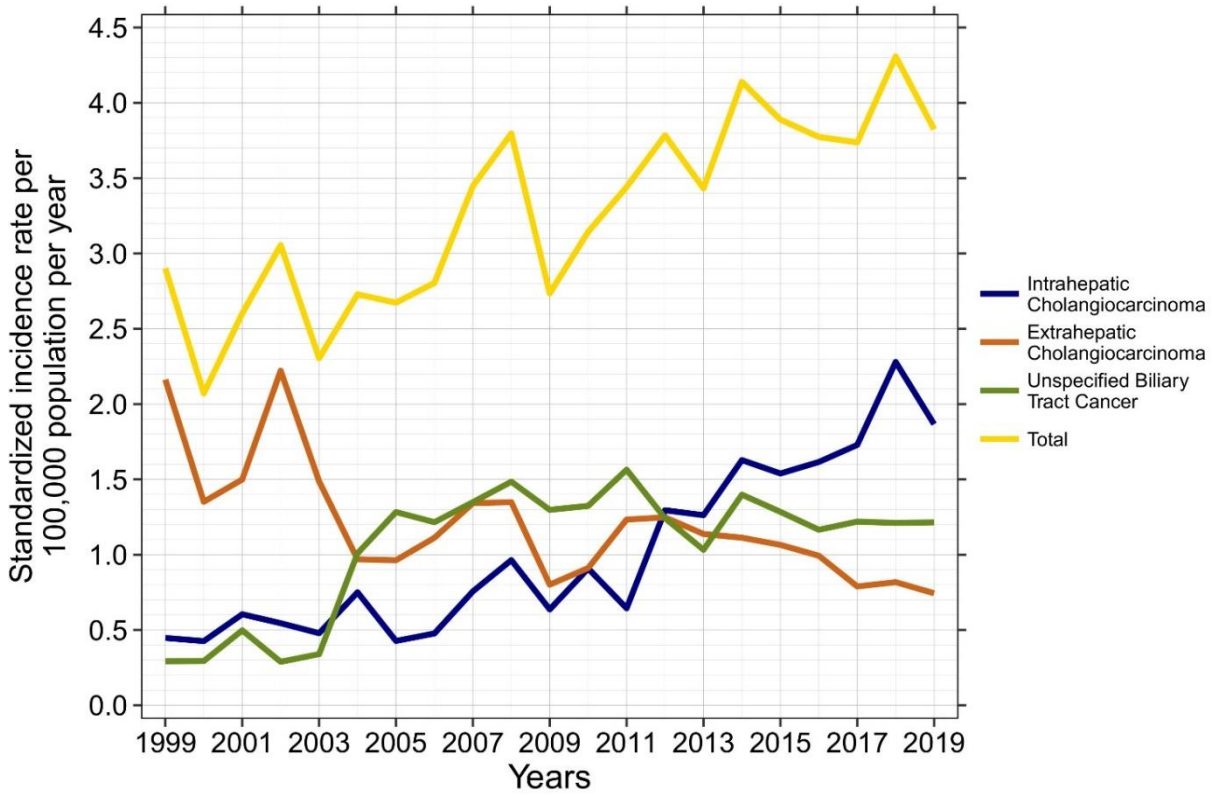
Background and Aims: Cholangiocarcinoma (CCA) is a usually fatal cancer originating from the biliary epithelium. Up-to-date data on incidence are crucial for our understanding of the disease, and therefore we set out to examine incidence of CCA in a nationwide Danish cohort.

Method: We included all 3,474 Danish patients with an ICD-10 diagnosis code of CCA (C22.1, C24.x) in the Danish Cancer Registry in 1999-2019. Patients were divided into intrahepatic (iCCA), extrahepatic (eCCA), and unspecified CCA based on ICD-O topography and morphology codes. We computed the standardized incidence rates per 100,000 person-years (SIR) of CCA by standardizing to the EU Standard Population 2013 and calculated incidence rate ratios (IRR) of SIRs for 2019 vs. 1999. We estimated annual change using a Poisson regression model.

Results: Of the 3,474 CCA patients, 47% were men. The median age of patients diagnosed in 1999 was 73 vs. 71 in 2019. For iCCA the median age rose from 68 to 71; for eCCA it fell from 72 to 70; and for unspecified CCA it fell from 75 to 71. The SIR for iCCA increased from 0.4 (95% confidence interval [CI] 0.2 to 0.6) in 1999 to 1.9 (95% CI 1.5 to 2.2) in 2019, yielding an IRR of 4.6 (95% CI 2.9 to 7.1) and a mean annual increase of 9.5% (95% CI 8.4 to 10.6). Between 1999 and 2019, the SIR for eCCA fell from 2.2 (95% CI 1.7 to 2.6) to 0.7 (95% CI 0.5 to 1.0), IRR: 0.4 (95% CI 0.3 to 0.5) and mean annual decrease -3.3% (95% CI -4.1 to -2.4), while the SIR for unspecified CCA rose from 0.3 (95% CI 0.1 to 0.5) to 1.2 (95% CI 0.9 to 1.5), IRR: 5.1 (95% CI 4.1 to 6.1) and mean annual increase 5.1% (95% CI 4.1 to 6.1). For total CCA, the IRR was 1.4 (95% CI 1.2 to 1.7) and the mean annual increase was 3.1% (95% CI 2.6 to 3.7) between 1999 and 2019.

Conclusion: The incidence of CCA has increased since 1999, driven by a 4-fold increase in incidence of iCCA between 1999 and 2019, while incidence of eCCA has decreased by half. Thus, iCCA is now far more common than eCCA whereas before 2010 it was the other way around. The reasons for this pattern are unclear, though part of the decrease in incidence of eCCA might be explained by an increase in incidence of unspecified CCA.

Figure:


Standardized incidence rate per 100,000 population per year
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1999 2001 2003 2005 2007 2009 2011 2013 2015 2017 2019
Years
Intrahepatic Cholangiocarcinoma
Extrahepatic Cholangiocarcinoma
Unspecified Biliary Tract Cancer
Total

P11-07
miRNome profiling analysis reveals novel hepatocellular carcinoma diagnostic, prognostic and treatment-related candidate biomarkers: post-hoc analysis of SORAMIC trail

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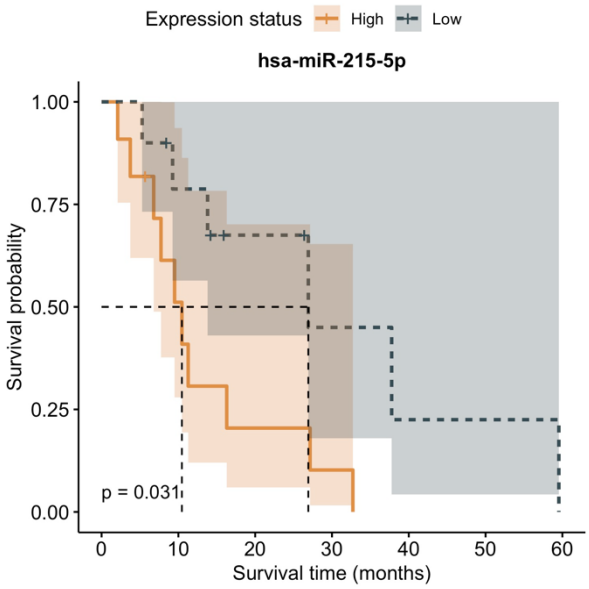
Background and Aims: Early diagnosis of hepatocellular carcinoma (HCC) as well as evaluation of prognosis and prediction of treatment efficacy for patients with HCC remain challenging due to the missing specific non-invasive biomarkers. The aim of this study is to identify HCC-specific microRNA (miRNA) pattern for diagnosis, prediction of prognosis and treatment response in patients with HCC.

Method: The study population included 42 HCC patients treated with different modalities within SORAMIC clinical trial: 22 patients received Sorafenib monotherapy, and 20 patients underwent ^{90}Y radioembolization in combination with Sorafenib. 20 individuals were included in the control group (CON) with their plasma samples collected. HCC patients underwent collection of plasma samples before and 7 to 9 weeks after the beginning of the treatment. Isolation of circulating miRNAs, preparation of small RNA sequencing libraries and next-generation sequencing were performed. Association analysis for novel diagnostic, prognostic and treatment-related candidate biomarkers was performed.

Results: A total of 42 differentially expressed (16 up-regulated and 26 down-regulated) miRNAs were identified comparing HCC baseline and CON plasma samples. In comparison to the baseline, sorafenib monotherapy was associated with lower level of hsa-miR-215-5p and hsa-miR-192-5p and increased concentration of miRNAs hsa-miR-483-5p and hsa-miR-23b-3p, while hsa-miR-215-5p was the sole down-regulated miRNA in comparison to the combinational therapy. Increased level of three miRNAs (hsa-miR-183-5p, hsa-miR-28-3p and hsa-miR-1246) was identified in non-responders versus responders after 7-9 weeks of sorafenib monotherapy. Kaplan-Meier survival analysis identified high hsa-miR-215-5p levels to be significantly associated with worse prognosis of HCC patients (see Figure).

Conclusion: Systematic miRNA profiling of highly characterized samples from SORAMIC clinical trial revealed a subset of potential miRNA biomarkers for diagnosis, prediction of prognosis and therapy response in HCC patients.

Figure:


Expression status
High
Low
hsa-miR-215-5p
1.00
0.75
0.50
0.25
0.00
Survival probability
p = 0.031
0
10
20
30
40
50
60
Survival time (months)

P11-08-YI
Secondary surveillance following curative treatment of hepatocellular carcinoma: a systematic review

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Background and Aims: Hepatocellular carcinoma (HCC) recurrence rates remain high and is a leading cause of death following curative treatments for HCC. Early detection and timely treatment of recurrence is the only potential option of improving survival of this patient cohort. However, in the absence of national or international recommendations, follow-up and secondary surveillance following curative intent treatment for HCC is conducted inconsistently. The aim of this systematic review was to determine whether secondary surveillance improves survival, and to inform on the optimal modality, frequency and duration of secondary surveillance.

Method: Electronic search was conducted on Ovid Medline, PubMed, EMBASE and Scopus for articles published up to October 2022 for studies that included patients who received curative treatment for HCC. Articles were screened and placed into categories: duration, modality, frequency and survival. PROSPERO registration: CRD42022334036

Results: Fifty-eight studies comprising 23,486 participants were included; only one study (n=734) reported on survival, 32 studies (n=4,304) reported on modality, 18 studies (n=14,563) reported on frequency and 8 studies (n=4,219) reported on surveillance duration – one study (n=334) reported on frequency and duration of secondary surveillance. The median duration of surveillance ranged from 1.9 months to 156 months. Of the 23,486 participants included in these studies 5,620 (24%) experienced HCC recurrence. The survival was significantly longer among those who underwent surveillance than those who did not (66.2 Vs. 20.2 months, p <0.01). Gadolinium enhanced MRI was a better diagnostic tool for HCC recurrence when compared to contrast enhanced CT. Similarly, the combination of tumour markers, alpha-fetoprotein (AFP) and des-gamma-carboxy-prothrombin (DCP), had a higher diagnostic yield for HCC recurrence than either tumour marker alone. The optimal frequency of surveillance was every 3–4 months for the first 3 years followed by every 6–12 months depending on the presence of risk factors for HCC recurrence. The pooled time to recurrence was within the first 33 months of curative treatment. However, studies suggest lifelong follow up of patients after curative treatment as survival has been shown to be more than 10 years for patients undergoing surveillance and early curative treatment of HCC recurrence.

Conclusion: Secondary surveillance of treated HCC is associated with improved survival. Available data suggest secondary surveillance with Gadolinium enhanced MRI and use of combination of tumour markers as optimal modality with more frequent surveillance in the first 3 years followed by less frequent surveillance thereafter. National and international guidelines should be updated with clear directions to clinicians on secondary surveillance.

P11-09
Landmark analysis of the risk of recurrence after resection or ablation for hepatocellular carcinoma: a nationwide study

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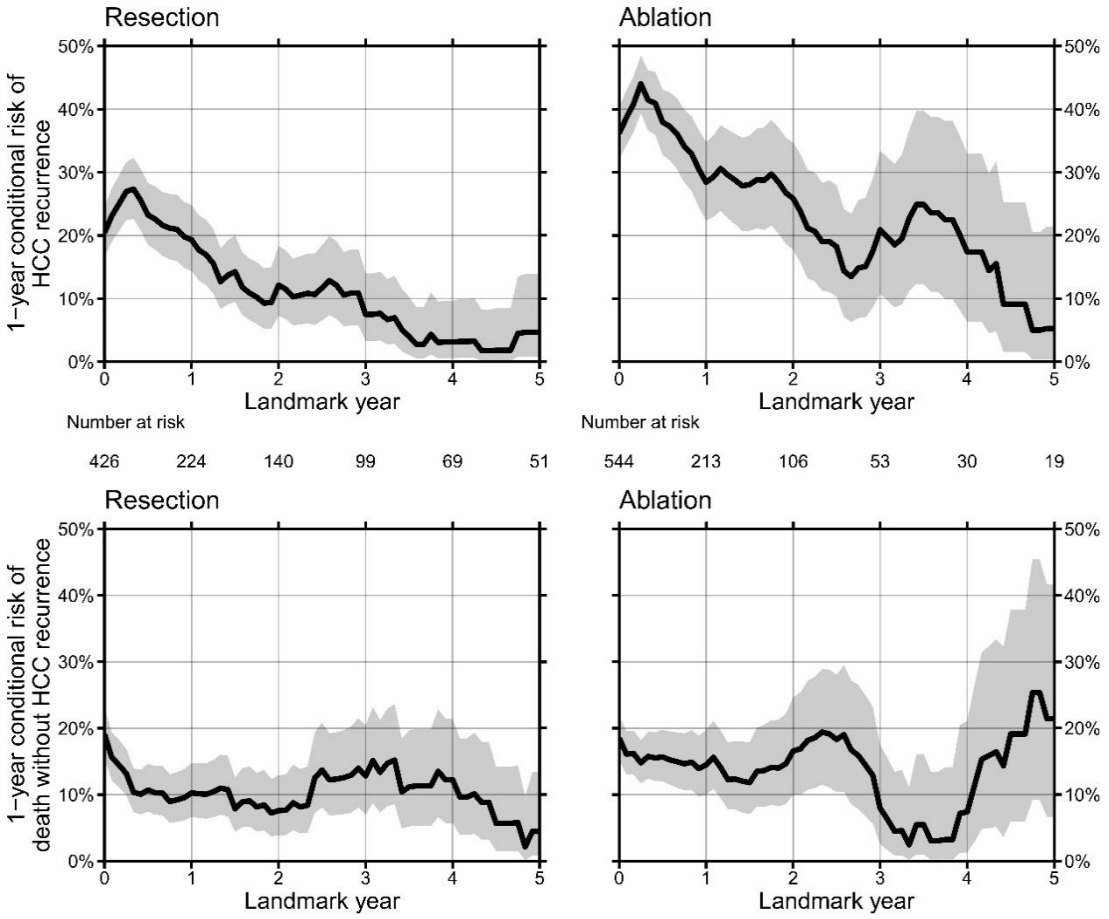
Background and Aims: The risk of hepatocellular carcinoma (HCC) recurrence at particular landmarks since the initial treatment is unknown. With this registry-based study, we aimed to provide a nuanced description of the prognosis following resection or ablation for HCC including landmark analyses.

Method: Using the Danish nationwide healthcare registries, we identified all patients who received resection or ablation in 2000-2018 as the first HCC treatment. HCC recurrence was defined as a new HCC treatment >90 days after the first treatment. We conducted landmark analyses of the cumulative risk of recurrence and of death without recurrence. Additionally, we computed the proportions of different causes of death over time.

Results: Among 4,801 patients with HCC, we identified 426 patients who received resection and 544 who received ablation. The two treatment cohorts differed in cirrhosis prevalence and tumour stage. The 5-year recurrence risk was 40.7% (95% confidence interval [CI] 35.5−45.8%) following resection and 60.7% (95% CI: 55.9−65.1%) following ablation. The 1-year recurrence risk decreased over the landmarks from 20.4% (95% CI: 16.6−24.6%) at the time of resection to 4.7% (95% CI: 0.9−13.9%) at the 5-year landmark. For ablation, the risk decreased from 36.1% (95% CI: 31.9−40.4%) at the time of treatment to 5.3% (95% CI: 0.4−21.4%) at the 5-year landmark (**Figure**). For both resection and ablation, 65% of deaths were caused by HCC.

Conclusion: The recurrence risk following resection or ablation for HCC is high from the treatment date, but it decreases greatly over the survival landmarks. This information is valuable for clinicians and their patients.

Figure:


Resection
Ablation
1−year conditional risk of HCC recurrence
50%
40%
30%
20%
10%
0%
0
1
2
3
4
5
Landmark year
Number at risk
426 224 140 99 69 51
544 213 106 53 30 19
Resection
Ablation
1−year conditional risk of death without HCC recurrence
Landmark year

P11-11
Features of patient who developed HCC after DAA treatment for hepatitis C virus (A Hospital Based and Cross Sectional Study)

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Background and Aims: The features of Hepatitis C virus patients with a sustained viral response (SVR), who developed HCC after DAAs (Direct-acting Antiviral Agents) therapy, are not very clear in our society.

Method: We studied population included 246 post DAAs SVR positive patients without history of pretreatment frequency, features risk factors of HCC, after end of treatment and mean follow up of 49 months were analyzed.

Results: Thirty two patients out of 246 (13%) developed HCC during a Median Observation period of 49 months. A comparative means analysis of patients with and without HCC, showed that age more than 45 years (<45=2/73, >45 30/173 = P <0.002), male sex (female 3/80, Male 29/176 < 0.002), child class (child A=12/218), (child class B & C 20/27 P = <0.005) and with advance stage of disease (chronic hepatitis 5/145, Compensated CLD 18/70, Decompensated CLD 10/31 P = <0.001) at the time of inclusion, post treatment deterioration of disease (Improved 13/216, Deterioration 19/30 P = <0.005) and patient belong to lower Sindh area (Lower Sindh area 25/142, born in upper Sindh 7/104 P = <0.04) were associate with HCC development. Pretreatment obesity, presence of diabetes, platelets less than 150, duration of disease since HCV diagnosis had no impact on development of HCC.

Conclusion:

1. Almost 13% of patient with HCC post DAAs developed HCC in mean follow up 49 months. (Equal to 4% per year HCC).
2. Advanced age, male sex, advance stage of liver disease, deterioration of disease after DAAs were risk factors for the development of HCC.
3. New risk factors that we found the patient belongs lower Sindh area are at increased risk of HCC development.
4. Limitation Hospital Based and Cross Sectional Study.

Figure:

P11-14
Carcinogenesis risk factors in cirrhotic patients with dysplastic nodules

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Background and Aims: Dysplastic nodules are encountered frequently in cirrhotic patients and are difficult to differentiate from early HCC. The risk for carcinogenesis is of paramount importance in determining the optimal time and procedure for the patients. The aim of the study is to evaluate the importance of biologic markers in predicting the risk of hepatocellular carcinoma (HCC) in patients with dysplastic nodules.

Method: We performed a retrospective observational single center study in a referral center for chronic liver diseases, including cirrhotic patients with newly diagnosed dysplastic nodules during January 2013-January 2018. The median follow-up period was 42.8 months. Dysplastic nodules were demonstrated by at least one imagining technique (contrast-enhanced ultrasonography CEUS, CT scan or magnetic resonance MR). Imagistic monitorization was performed every 6 months for a duration of at least 12 months. We noted patients' age and gender, liver disease etiology, Child-Pugh score, Model for End-stage Liver Disease score (MELD), ALBI score, liver function tests, platelets, C-reactive protein (CRP), alpha-fetoprotein (AFP) and nodule characteristics: number, size (mm), location. The primary endpoint was malignant transformation of the dysplastic nodules.

Results: We included 108 patients with a total of 164 dysplastic nodules. Mean age in the study group was 57.92 +/- 31.03 years. Study lot distribution according to cirrhosis etiology was: 13 patients with HBV + HDV infection, 31 patients with metabolic liver disease, 41 patients with chronic HCV infection and 23 patients with chronic HBV infection. Transformation in HCC occurred in 59 nodules (35.97%). Increased levels of liver function tests, CRP and AFP, high MELD, Child and ALBI scores, HBV (with or without HVD infection) were HCC risk factors. Notably, nodules located in the left liver lobe were more prone to carcinogenesis.

Conclusion: Persistent inflammation expressed by high values of liver function tests, as well as advanced liver disease and the presence of dysplastic nodules in the left lobe are associated with increased risk for HCC and require close monitoring or prophylactic interventions

Figure: Table 1. Risk factors for carcinogenesis in univariate Cox regression analysis.

Variable	Hazard ratio	95% CI	p
Age (years)	0.83	0.67-1.45	0.53
Etiology: HBV	3.26	2.29- 4.12	0.03
Etiology: HBV + HDV	5.34	3.56 – 7.24	0.01
ALT (U/L)	1.34	1.07-1.69	0.04
AST (U/L)	2.06	1.54- 2.76	0.03
GGT (U/L)	2.74	1.96-3.13	0.02
AlkP (U/L)	2.56	2.11- 3.01	0.03
INR	1.78	1.16-2.13	0.04
CRP (mg/L)	3.17	2.79 – 3.84	0.03
AFP (ng/ml)	1.42	1.12-1.87	0.04
Plateles ($x10^3/mm^3$)	1.22	1.03-1.67	0.05
Child score (per unit increase)	2.04	1.82- 2.31	0.02
MELD (per unit increase)	3.28	2.57- 4.11	0.02
ALBI: 3	7.23	5.92 – 14.24	<0.001
Nodule location: left lobe	4.35	4.01 – 8.24	<0.001

P11-15
A prospective study for validation of General Evaluation Score for hepatocellular carcinoma risk stratification in chronic hepatitis C patients

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Background and Aims: We developed and both internally and externally validated a simple scoring system called General Evaluation Score (GES) for HCC risk stratification.

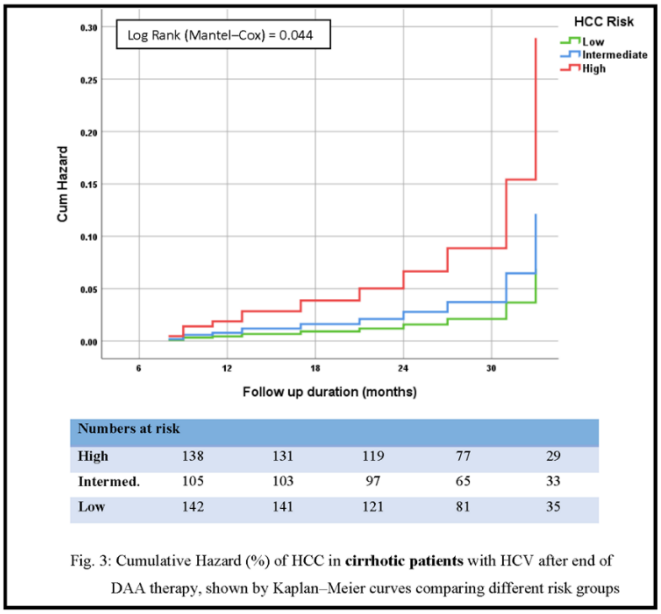
To ascertain the validity of this score in a large prospective cohort of cured hepatitis C patients with compensated advanced chronic liver disease who achieved a sustained virological response following direct acting antivirals.

Method: This single-center prospective study included 463 consecutive patients, with advanced fibrosis (≥F3) who achieved SVR. The patients were recruited from the outpatient clinics at the Egyptian Liver Research Institute and Hospital between January 2018 and October 2019. All patients underwent abdominal ultrasound and multislice computed tomography for surveillance of HCC before starting antiviral therapy. Patients were followed up every 6 months after the end of treatment using ultrasonography and alpha-fetoprotein in addition to MSCT every 12 months.

Results: A total of 463 patients were included, of which 197 (42.5%), 114 (24.6%), and 152 (32.8%) had low-, intermediate-, and high-risk scores calculated before treatment, respectively. HCC incidence rate was 2.61/100 py (95% CI = 1.73–3.80); 25 cases developed HCC during follow-up. The incidence of HCC was 0.97% (95% CI: 0.31–2.34), 1.68% (95% CI: 0.53–4.05), and 5.57% (95% CI: 3.35–8.74) in the low, intermediate, and high-risk groups, respectively. The HCC incidence increased significantly with higher scores ($p < 0.001$). Harrell's c-statistic for this model was 0.728.

Conclusion: This prospective study demonstrated the ability of GES to predict HCC occurrence and accurately stratify patients into low-, intermediate-, and high-risk groups.

Figure:



Numbers at risk					
High	138	131	119	77	29
Intermed.	105	103	97	65	33
Low	142	141	121	81	35

Fig. 3: Cumulative Hazard (%) of HCC in **cirrhotic patients** with HCV after end of DAA therapy, shown by Kaplan–Meier curves comparing different risk groups

P11-16
Influence of the sustained viral response (SVR) in patients with cirrhosis due to hepatitis C virus (HCV) and diagnosis of hepatocellular carcinoma (HCC)

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Background and Aims: In patients with chronic HCV infection, achieving SVR reduces the risk of HCC. The objective of our study was to evaluate the presentation of HCC in patients with chronic HCV according to the status of the infection (SVR vs not SVR) and their survival.

Method: Single-center prospective study with all consecutive cases of HCC secondary to chronic HCV infection registered between June 1, 2015 and August 31, 2022. Baseline characteristics, first treatment applied and survival were evaluated, censored at the end of follow-up, date of transplant or death. Patients were classified into 2 groups, with SVR and without SVR at the time of HCC diagnosis.

Results: 280 patients were included, 75% male, median age 63.4 years, 81% Child A, 12% Child B. 60% were diagnosed within the screening program and 51.8% at very early/early stage (BCLC-0/A). The median overall survival was 24 months. The median time between SVR and HCC diagnosis was 31 months (IQR 8-53). 55.4% (n=155) were diagnosed with SVR and 125 (44.6%) without SVR. In 37.6% of the patients without SVR, HCC and chronic liver disease were diagnosed at the same time. Patients with SVR are younger (61.7 vs 65.5; p=0.001), diagnosed more frequently within the screening program (74.2% vs 42.4%; p<0.001) and at the BCLC-0/A stages (62.5% vs 38.4%, p < 0.0001). Likewise, they were treated either with liver transplantation (14.2% vs 3.2%) or thermoablation (16.1% vs 13.6%) and less frequently they remained in natural history after diagnosis (16.8% vs 41,6% p=0.0001). Patients with SVR have better liver function estimated by the ALBI index (ALBI-1 64.3% vs 34.1%, p<0.001)). Overall survival was significantly higher in patients with SVR (median 40 months, 95% CI 35,84-44,15) compared to patients without SVR (median 16 months, 95% CI 9,47-22,52) (p=0.001). Baseline factors independently associated with survival were the presence of SVR (p=0.038), biannual screening (p=0.005), ALBI index (p=0.001), and BCLC stage (p=0.0001).

Conclusion: In our environment there is still a significant number of patients in whom HCC is the first manifestation of chronic HCV infection. In those in which HCC appears after reaching SVR, staging is more initial and therapeutic options and survival are significantly better.
REF: Kanwal F, Kramer J, Asch SM, Chayanupatkul M, Cao Y, El-Serag HB. Risk of Hepatocellular Cancer in HCV Patients Treated With Direct-Acting Antiviral Agents. Gastroenterology. 2017;153(4):996-1005.

P11-17
Inflammation and immune-based prognostic scores as prognostic biomarkers for HCC treatment: A single-center study in the last decade

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Background and Aims: Hepatocellular carcinoma (HCC) is one of the leading cancer-related causes of death worldwide. The pathophysiology of HCC is complex and multifactorial. However, Systemic inflammation has a pivotal role in HCC development and progression. Hence, in this study, we aimed to determine the impacts of inflammation and immune-based prognostic scores to predict the survival and progression of HCC.

Method: In this retrospective study, HCC patients confirmed with biopsy or MRI were enrolled from January 2010- December 2020. We evaluated the impact of baseline alpha-fetoprotein (AFP), CALLY Index (CRP-albumin-lymphocyte index), Systemic immune-inflammation index (SII), ALBI score, and AST/ALT ratio (AAR) on survival, vascular invasion, extrahepatic spread, and treatment responses. Univariate and multivariate logistic analyses were used to determine the independent factors, and the receiver operating characteristic (ROC) curves were performed to evaluate the predictive performance. The area under receiver operating characteristic curve (AUROC) was used to assess the performance of the markers.

Results: A total of 199 HCC patients were divided into a curative (n=44) or non-curative (n=145) treatment group according to the radiologic presence of the HCC in the last outpatient control. The difference in AFP, CALLY Index, AAR, SII, and ALBI scores for the non-curative HCC group were statistically significant ($p < 0.05$). Area under the curves (AUCs) of them for predicting non-curative HCC were 0.651, 0.649, 0.636, 0.625, 0.609, with corresponding sensitivities of 48.7%, 44.3%, 59.8%, 72.5%, 60.8%, and specificities of 84%, 89.5%, 70.5%, 50%, 61.4%, respectively. AFP, CALLY Index, AAR, SII, and ALBI score (odds ratio (OR):5,018, %95 confidence interval (CI): 2,108-11,947; OR: 6,753, %95 CI: 2,266-20,124; OR: 2,948, %95 CI: 1,461-5,948; OR:2,405, %95 CI: 1,204-4,806; OR:2,675, %95 CI: 1,342-5,331) were found to be independent predictive factors. In addition, the results were correlated with the assessment of the last tumor size, vascular invasion, and metastasis in the non-curative treatment group. The mean survival duration was 62±5 months. Patients with high AFP, SII, and ALBI Scores (p=0,001; p<0,01), p=0.042; p<0,05, p=0,001; p<0,01), and a low CALLY Index (p=0,001; p<0,01) had higher mortality rates. However, AAR was not associated with overall survival.

Conclusion: The results of our single-center study verified that baseline AFP, CALLY Index, AAR, SII, and ALBI scores could be a powerful tool and prognostic factor for predicting the prognosis of patients with HCC. Because these indexes are practical and easy to obtain, these measurements should be regarded as biomarkers in the clinical management of HCC.

Figure:

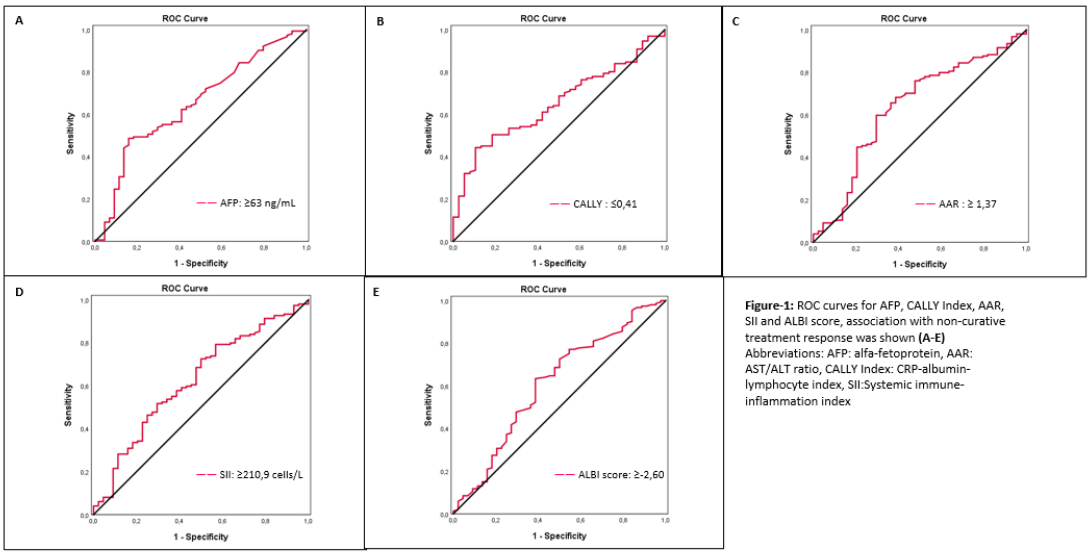

Figure-1: ROC curves for AFP, CALLY Index, AAR, SII and ALBI score, association with non-curative treatment response was shown **(A-E)**
Abbreviations: AFP: alfa-fetoprotein, AAR: AST/ALT ratio, CALLY Index: CRP-albumin-lymphocyte index, SII:Systemic immune-inflammation index

P11-19
Epidemiology of hepatocelullar carcinoma in transylvania

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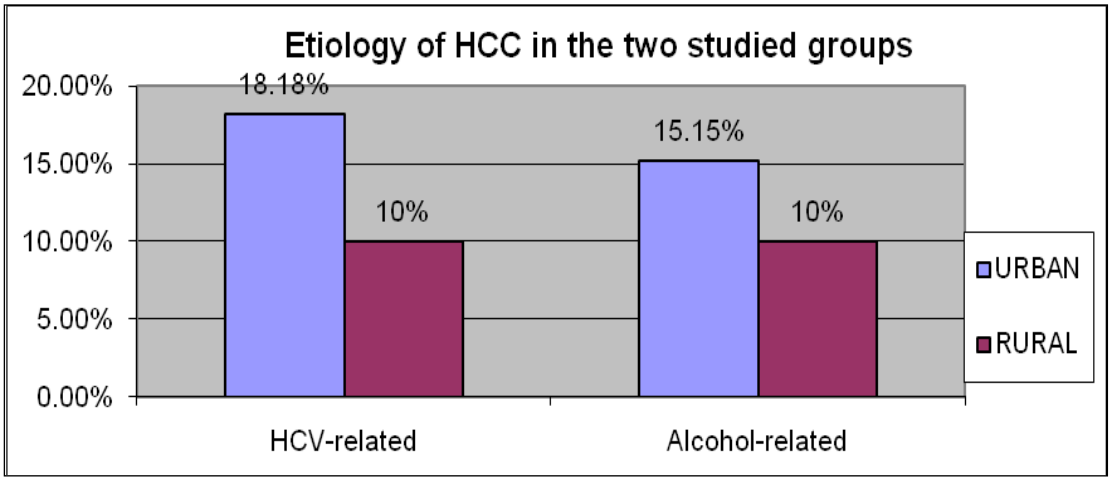
Background and Aims: Few studies have been done in order to establish the epidemiology of the hepatocellular carcinoma (HCC) in Transylvania. Our aim was to study which is the prevalence and other epidemiological features of the HCC among the hospitalized patients from Southern Transylvania.

Method: We have performed a retrospective study on a 7 years period of observation. From the total of 7224 patients who were hospitalized on the Gastroenterology Department of the Clinical County Hospital from Sibiu, 80 were diagnosed with HCC. We the divided the HCC patients into two groups regarding the provenience area: urban or rural.

Results: The HCC prevalence was 1.1% from the hospitalized patients. 75% from them are from urban areas. The medium age of the patients from rural areas was 63 years, comparing with 64.64 years at those from urban areas (p=0.3677). The HCC incidence in chronic liver disease of various aetiologies was also different between the two groups: HHC affected patients with hepatitis C virus more in urban areas than in rural (18.18% comparing with 10%). 15.15% from the HHC aetiologies in the urban areas were alcohol-related, versus only 10% in the rural areas. Also, there was observed that the nodules which were seen by one imaging technique (ultrasound or computer tomography) had different sizes in the two groups. The medium size of the liver lesions at the patients from urban areas was 6.782 cm, comparing with only 4.785 cm at the patients from rural areas, the difference being statistically significant (p=0.0457). Between the two groups there were also significant differences regarding the level of serum cholinesterase, these being higher in the urban group of patients (p=0.089). The rest of the laboratory parameters did not differ significantly between the two groups: the level of hepatic cytolysis (p=0.47 for aspartate-aminotransferase, respectively p=0.35 for alanin-aminotransferase), total bilirubine level (p=0.37), gamma-glutamyl transpeptidase (p=0.044), the number of platelets (p=0.118), the coagulation parameters (p=0.20 for prothrombine time), the level o cholesterol (p=0.47) and triglycerides (p=0.21).
Conclusion: The prevalence of the HCC among the hospitalized patients from Southern Transylvania is about 1.1%. There are significant geographic differences in HCC incidence. The majority (75%) of the patients diagnosed with hepatocellular carcinoma live in urban areas. In urban areas, HCC affects older patients with hepatitis C virus or alcohol-related cirrhosis. Also, the liver lesions' dimensions are different between these two geographical areas; in urban areas the nodules have, when diagnosed, higher sizes than in rural areas, so, the prognosis may be poorer in urban areas.

Figure:


Etiology of HCC in the two studied groups
20.00%
15.00%
10.00%
5.00%
0.00%
18.18%
10%
15.15%
10%
HCV-related
Alcohol-related
URBAN
RURAL

POSTER ABSTRACT PRESENTATIONS

Nurses & AHPs

P02-04
Value based Healthcare: Patient perception of the liver cancer Advanced Practice Nurse care at the Barcelona Clinic Liver Cancer

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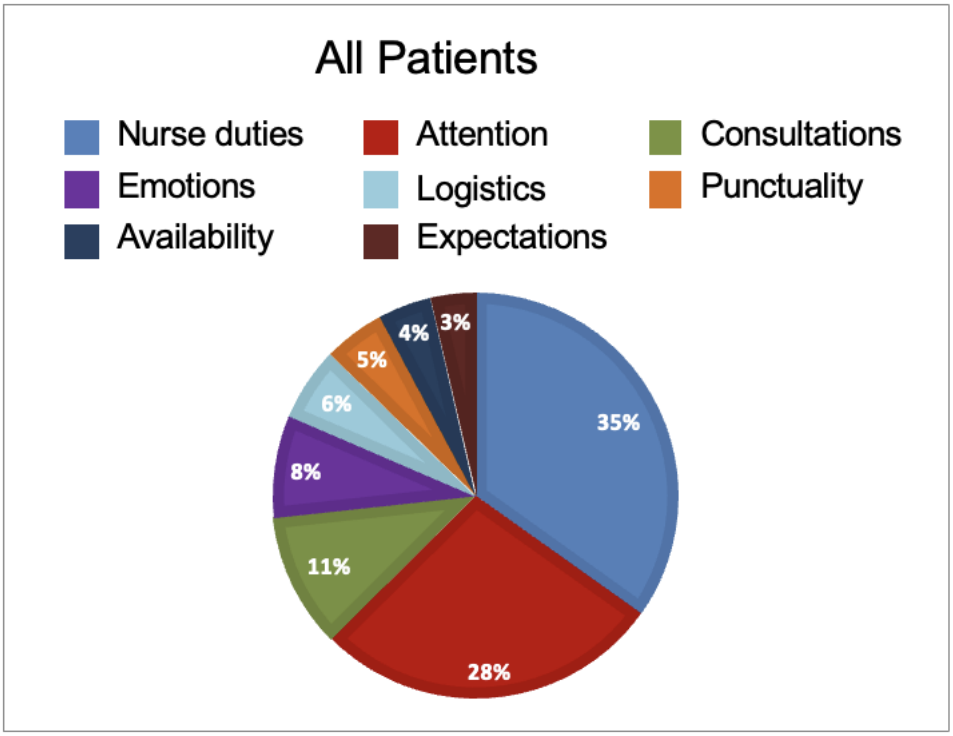
Background and Aims: Management of hepatocellular carcinoma (HCC) was traditionally based on the treatment's safety and efficacy. However, patient experience, which is the third pillar of the quality of Health Care was less evaluated. This study assesses the patient valued-heath care areas and experience of the liver cancer patients in the outpatient clinic at the BCLC. The interview was based on the patients' experience with the Advanced Practice Nurse (APN) program at BCLC.

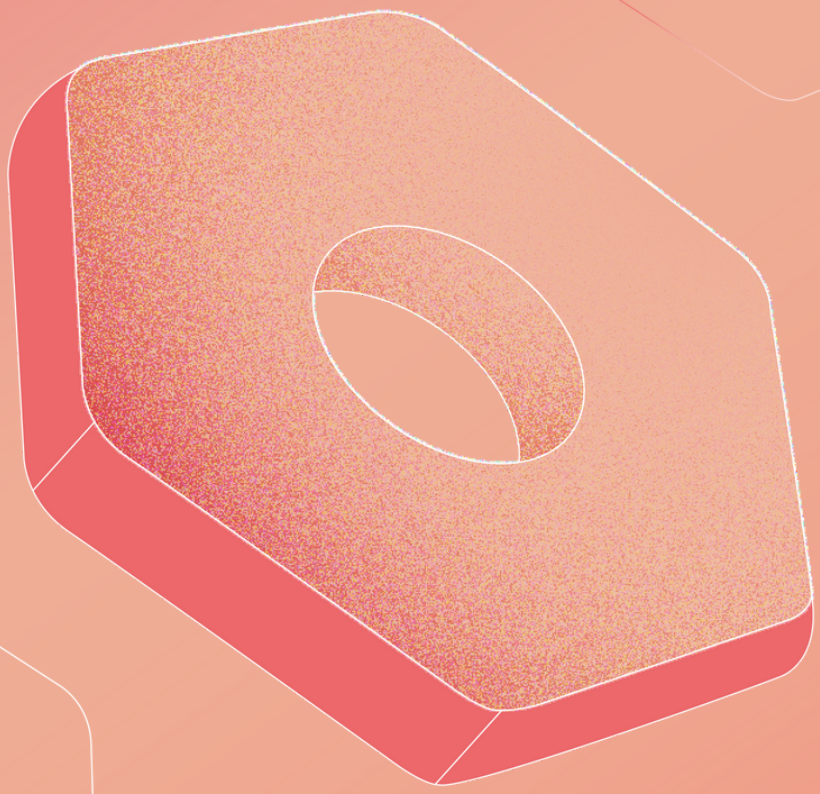
Method: Liver cancer patients included in APN-led-educational programs were recruited and were purposefully selected based on gender, age and disease stage. Individual interviews were conducted between September and October 2020. From September to October, a qualitative study through interview techniques were carried out. The interviews, which lasted on average 22.4 minutes, (Range 13-50) were audio-recorded and transcribed verbatim and analyzed by MAXQDA software. Patients were divided into two groups according to their knowledge of the APN program (with and without knowledge)

Results: Twenty-one interviews were performed. From the transcription analysis, 306 considerations were obtained which were grouped into 34 categories and 8 meta-categories (Figure 1). Although **'Nurses' duties and 'humanely'** were the main themes in all patients, other different topics were in the top-third category in each group: 'Expectations' in patients without knowledge and 'emotions' in patients who were already familiar with the APN program. Under **'emotions'** some patients mentioned that nurses bring peace of mind, reassurance, others nervousness, fear and some patients commented that they felt alone during admission. Regarding **'expectations'**, the patients mentioned that what they expected from the nurses is to be treated well and professionalism, which was the case. Surprisingly, punctuality (4 % and 5% in patients with and without previous contact with BCLC-APN; respectively) and administrative support (6% in both groups) were less mentioned than the nurses expected.

Conclusion: In qualitative studies results are not generalizable to all patients. Their needs/priorities can be interpreted as areas of the BCLC-APN program which need to be covered or the reflection of patients' needs according to their knowledge of the program. These results are the rationale for developing a Value Based-APN program which includes the patients' perceptions.

Figure:


All Patients
Nurse duties
Attention
Consultations
Emotions
Logistics
Punctuality
Availability
Expectations
35%
28%
11%
8%
6%
5%
4%
3%



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