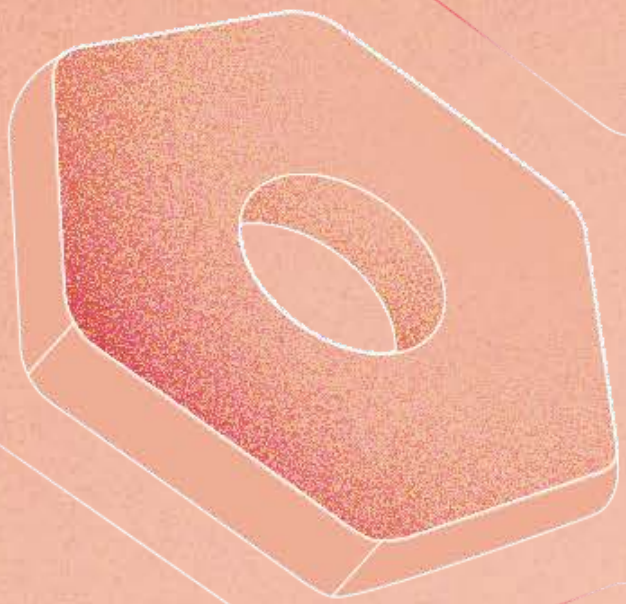
LIVER CANCER SUMMIT 2022

3-4 FEB
ONLINE



Abstract book

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OS-35
Cholangiocytes are predisposed, but not committed, to malignant transformation following primary cilia loss

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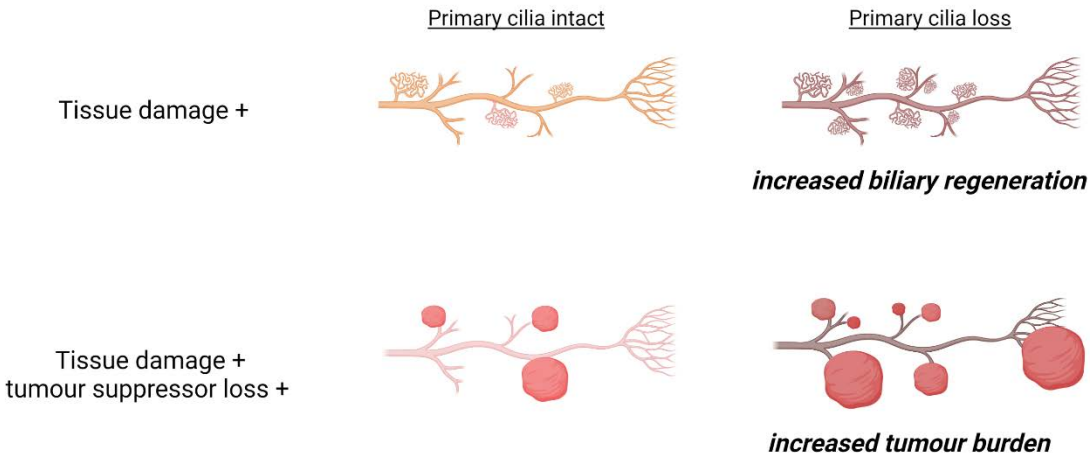
Background and aims: Primary cilia (PC) are sensory organelles that project from the apical membranes of cholangiocytes and function to coordinate several signalling pathways. Loss of PC in human intrahepatic cholangiocarcinoma (ICC) has previously been reported and PC have been described as tumour suppressor organelles. However, little is known of the downstream processes that follow PC loss and it remains unclear as to whether the loss of this organelle alone is sufficient to initiate tumorigenesis.

Method: We generated a mouse model to allow for conditional deletion of essential PC gene Wdr35 from the adult biliary tract. This mouse line was: (1) induced to delete PC alone from the biliary tract; (2) administered hepatotoxin thioacetamide (TAA) to model PC loss on the background of liver injury; and (3) crossed with a transgenic murine line that allows for Trp53 and Pten deletion in the biliary tract to model ICC when administered TAA. We performed single cell and bulk RNA sequencing on tissues from these disease models.

Results: Following loss of PC alone, polycystic livers develop. We identified the upregulation of numerous oncogenic-relevant signalling genes on cystic epithelia, such as those of the Mitogen Associated Protein-Kinase (MAPK) and Transforming Growth Factor Beta (TGFβ) pathways. Yet, despite these oncogenic signatures, ducts lacking PC do not develop ICC tumours within 1 year. When PC loss is combined with liver injury, there is an increase in the formation of expansive ductular lesions which stretch between portal-tracts compared to injury alone, suggesting that PC restricts biliary regeneration and their loss drives rapid expansion of the biliary tree. To test whether loss of PC is additive in ICC and promotes more rapid tumorigenesis, we deleted PC in tandem with tumour suppressor genes Trp53 and Pten. Livers lacking PC have a significantly increased tumour burden compared to Trp53-/- and Pten loss alone. RNA sequencing reveals tumour cells without PC downregulate immunoregulatory genes, indicating that PC loss allows tumour cells to maintain a cold immune microenvironment and implicate the primary cilium in the immunomodulatory landscape of ICC.
Conclusion: PC loss in the adult biliary tract results in the development of polycystic livers that harbour oncogenic molecular signatures, but do not form macroscopic tumours. Cystic epithelia could therefore serve as premalignant lesions that are prone to malignant transformation upon tissue damage or second-hit genetic mutations.

Figure:



OS-56

Neural features of hepatocellular carcinoma, implications for tumour stratification, and identification of a pertinent animal model

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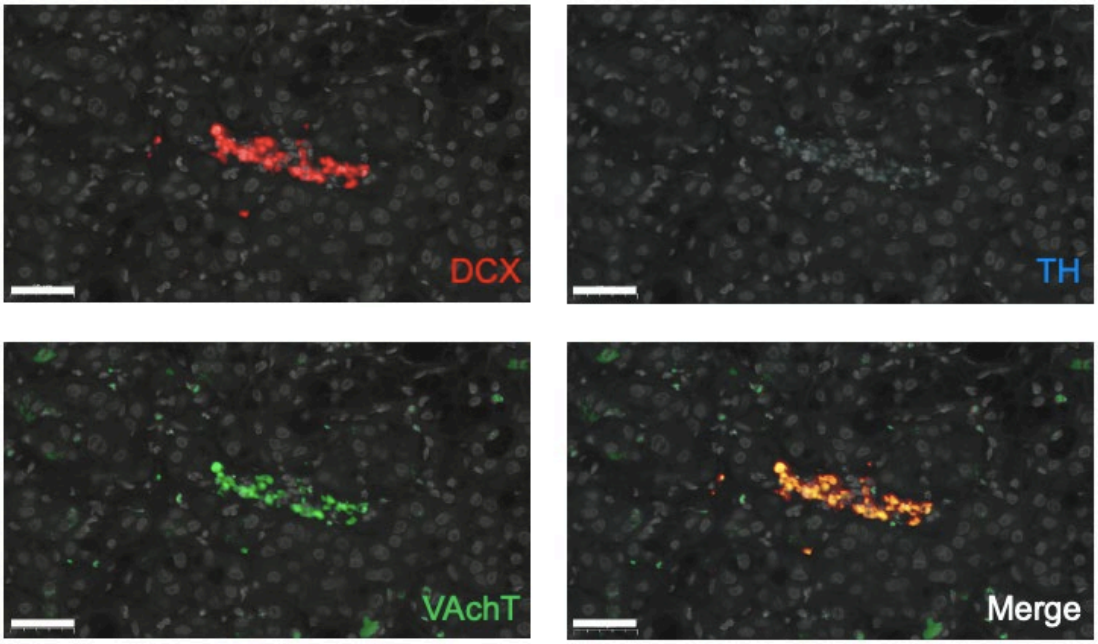
Background and aims: The aggresiveness and the unexplained interpatient variation of hepatocellular carcinoma (HCC) onset and clinical outcome remain a major challenge for clinicians. Here, we focused on the under-explored link between the disease and the hepatic autonomic nervous system (ANS), that is unique to each patient and prone to be targeted by approved and well characterized human drugs.

Method: Conventional biochemistry, sample imaging, and bioinformatics were used.

Results: HCC samples (French Liver Biobank) were shown to host mature and progenitor nerves in tumour capsules and fibrotic areas, but also in tumour bulks (WB: n=51, multiplex IHC: n=24), These nerves were of parasympathetic orientation. We have identified an important regulation of several adrenergic and, more intensely, cholinergic receptors transcripts in paired cirrhosis / HCC samples (n=160). Using the TCGA HCC dataset (n=193), we then defined an HCC neural signature, derived from all adrenergic and cholinergic receptors transcripts levels, that allowed stratification into two groups, called neuroclasses thereof. The first is composed of samples with adrenergic orientation, and associates with better prognosis tumours than the second, featured by cholinergic enrichment, that tightly associates with poor prognosis tumours or functions (i.e AFP-rich, proliferative tumours). Cholinergic tumours were linked namely to mitotic functions, TGF-β, EMT, Ras, and Akt/mTOR pathways, and 18 poor prognosis GSEA pathways. The only found biological correlate of either group was the *CTNNB1* mutational status (p=0.05), suggesting that HCC neuroclasses enrich the current classification scheme. Immune functions (including PD-1 pathway) were enriched in adrenergic tumours while also associated with weaker statistical parameters in the cholinergic neuroclass. This neurosignature was found to bear better predictive value (p=0.02 and 0.03 for OS and PFI) in favor of adrenergic samples. Finally, we identified that a cirrhotic, DEN-treated, rat model of HCC exhibits liver neurogenesis of parasympathetic orientation that is synchronous to cancer onset, suggesting its pertinence for in vivo studies.

Conclusion: Altogether, such data identify neurogenesis in HCC, and suggest that the parasympathetic branch of the ANS may influence the pathobiology of the disease. They also argue for the consideration of ANS targeting drugs, of which many are clinically safe and well characterized, in HCC studies.

Figure: Infiltration of tumour bulk by immature neurons of parasympathetic orientation



OS-123
Dynamic organotypic culture of primary liver cancer as a personalised immunocompetent drug screening platform for immuno-oncology

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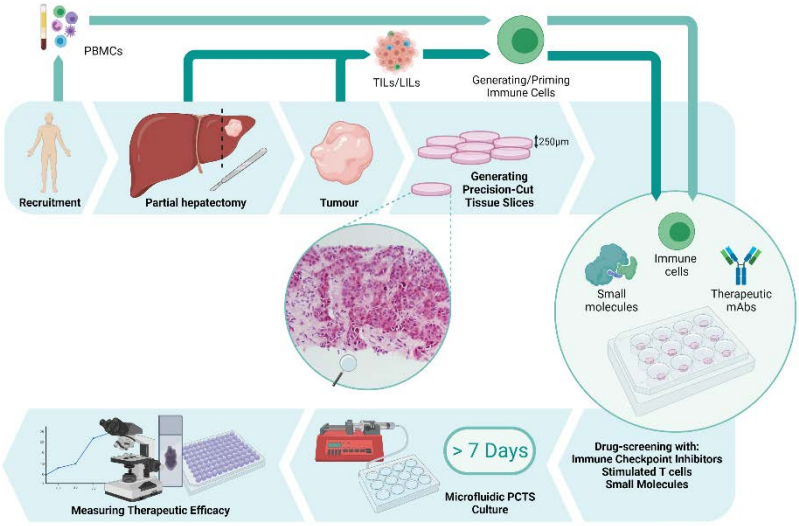
Background and aims: Current primary liver cancer (PLC) models fail to truly encompass the human tumour immune microenvironment, exacerbating a recognised discord between the preclinical and clinical successes of emerging (immuno)therapeutics. The organotypic culture of 3D human Precision Cut Tumour Slice (PCTS) is a cancer explant model which retains tumour specific histoarchitecture, aetiological background, disease phenotype, resident immune landscape, and checkpoint (CP) expression for up to 7 days ex-vivo. Our study aims to (1) validate PCTS to assess patient specific therapeutic responses and (2) advance culture conditions using a proprietary Multi-well Plate (MuPL) perfusion bioreactor to extend PCTS lifetime and allow perfusion of immune cells through PCTS.

Method: PCTS generated from PLC (HCC & CCA) were treated with approved single agent or combinatory checkpoint inhibitor (CPI), monoclonal antibody (mAb) or kinase inhibitor (KI) therapy for up to 7 days. Therapeutic response was determined by evaluating histology (H&E and Sirius red), apoptosis/cell death (TUNEL, lactate-dehydrogenase release, cytokeratin 18), and proliferative capacity (PC; Ki67). Gene expression was assessed using QuantiGene RNA Assay. Resident immune cells were assessed by immunofluorescence and FACS. In addition, PCTS/immune cells co-cultures in the MuPL bioreactor were longitudinally assessed for viability, histology, and tissue integrity.

Results: Doxorubicin was used as a positive cell death control in all treated patients, decreasing PCTS viability and PC by day5. Compared to monotherapy, nivolumab + regorafenib therapy decreased the tumour to stroma ratio and PC in all patients by day7. Also, significantly increased apoptosis was detected in one patient, who comparatively showed higher CP expression including PD-1, PD-L1 and CTLA-4. Other combinatorial immunotherapies, including atezolizumab + bevacizumab and nivolumab + ipilimumab, reduced PC without affecting histology or viability. Overall immunotherapeutic response was patient specific. Additionally, PCTS and immune cells co-cultured in the MuPL bioreactor maintained viability, structural integrity and histoarchitecture for >7 days.

Conclusion: PCTS can be used as a powerful tool to study personalised responses to (immuno)therapeutics. In addition, PCTS can be successfully cultured in a perfusion system, recapitulating tumour-immune cell interactions, allowing assessment of response to cell and vaccine therapy ex-vivo.

Figure:


PBMCs
TILs/LILs
Generating/Priming Immune Cells
Recruitment
Partial hepatectomy
Tumour
250µm
Generating Precision-Cut Tissue Slices
Immune cells
Small molecules
Therapeutic mAbs
Drug-screening with:
Immune Checkpoint Inhibitors
Stimulated T cells
Small Molecules
> 7 Days
Microfluidic PCTS Culture
Measuring Therapeutic Efficacy

OS-125
Targeting NAE1-mediated protein hyper-NEDDylation halts cholangiocarcinogenesis and impacts on tumour-stroma crosstalk in experimental models

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

Method: levels and function of NEDDylation, together with response to Pevonedistat (NEDDylation inhibitor) or CRISPR/Cas9 against *NAE1* were evaluated *in vitro*, *in vivo* and/or in patients with CCA. Development of preneoplastic lesions in *Nae1*$^{+/-}$ mice was investigated using an oncogene-driven CCA model. The impact of NEDDylation in CCA cells on tumour-stroma crosstalk was assessed using CCA-derived cancer-associated fibroblasts (CAFs). Proteomic analyses were carried out by mass spectrometry.

Results: NEDDylation machinery was found overexpressed and overactivated in human CCA cells and tumours, correlating with poor prognosis. Most NEDDylated proteins found upregulated in CCA cells, after NEDD8-immunoprecipitation and further proteomics, participate in cell cycle, proliferation or survival. Genetic (CRISPR/Cas9-*NAE1*) and pharmacological (pevonedistat) inhibition of NEDDylation reduced CCA cell proliferation and impeded colony formation *in vitro*. NEDDylation depletion (pevonedistat or *Nae1*$^{+/-}$ mice) halted tumorigenesis in subcutaneous, orthotopic, and oncogene-driven models of CCA *in vivo*. Moreover, pevonedistat potentiated chemotherapy-induced cell death in CCA cells *in vitro*. Mechanistically, impaired NEDDylation triggered the accumulation of cullin RING ligase or NEDD8 substrates, inducing DNA damage and cell cycle arrest. Furthermore, NEDDylation impairment in CCA cells reduced the secretion of proteins involved in fibroblast activation, angiogenesis, and oncogenic pathways, ultimately hampering CAF proliferation and migration.

Conclusion: aberrant protein NEDDylation contributes to cholangiocarcinogenesis by promoting cell survival and proliferation. Moreover, NEDDylation impacts the CCA-stroma crosstalk. Inhibition of NEDDylation with pevonedistat may represent a potential therapeutic strategy for patients with CCA.

OS-37

Cholangiocarcinoma landscape in Europe: diagnostic, prognostic and therapeutic insights from the ENSCCA Registry

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Background and aims: Cholangiocarcinoma (CCA) is a rare and heterogeneous biliary cancer, with increasing incidence and related mortality. This study investigates the clinical course of CCA and subtypes (intrahepatic (iCCA), perihilar (pCCA), and distal (dCCA)) in a pan-European cohort.

Method: The ENSCCA Registry is a multicentre observational study. Patients with histologically-proven CCA diagnosis between 2010-2019 were included. Demographic, histomorphological, biochemical, and clinical studies were performed.

Results: Overall, 2,234 patients were enrolled (male:female=1.29). iCCA (n=1,243) was associated with overweight/obesity (58.5%) and chronic liver diseases involving cirrhosis (12.6%) and/or viral hepatitis (10.4%); pCCA (n=592) with primary sclerosing cholangitis (8.8%); and dCCA (n=399) with choledocholithiasis (10.3%). At diagnosis, 42.2% of patients had local disease, 29.4% locally-advanced disease (LAD), and 28.4% metastatic disease (MD). Serum CEA and CA19-9 showed low diagnostic sensitivity (69.1% and 40.9% below cutoff, respectively), but their concomitant elevation was associated with increased risk of presenting with LAD [OR=2.16;95%CI:1.43-3.27] or MD [OR=5.88;95%CI:3.69-9.25]. Patients undergoing resection (50.3%) showed the best outcome, particularly with negative-resection margin (R0) [median overall survival (mOS)=45.1 months]; however, margin involvement (R1) [HR=1.92;95%CI:1.53-2.41;mOS=24.7 months] and lymph node invasion [HR=2.13;95%CI:1.55-2.94;mOS=23.3 months] compromised prognosis. Among patients with unresectable disease (49.6%), the mOS was 10.6 months for those receiving active palliative therapies, mostly chemotherapy (26.2%). Patients receiving best supportive care (20.6%) had mOS of 4.0 months, with iCCAs showing worst outcome compared to p/dCCAs. ECOG performance status [HR=1.52;95%CI:1.01-2.31], MD [HR=4.03;95%CI:1.82-8.92] and CA19-9 [HR=2.79;95%CI:1.46-5.33] were independently prognostic for OS.

Conclusion: CCA is still diagnosed at advanced stage, a proportion of patients fail to receive cancer-specific therapies, and prognosis is dismal. Identification of preventable risk factors and implementation of surveillance in high-risk populations are required to decrease cancer-related mortality.

OS-55

Regorafenib in patients with unresectable hepatocellular carcinoma in routine clinical practice: Exploratory analysis of safety and overall survival in the prospective, observational REFINE study

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Background and aims: Regorafenib is globally approved for the treatment of patients with uHCC after prior sorafenib. An interim analysis of the observational REFINE study supports the safety and effectiveness of regorafenib in patients with uHCC in real-world practice (Lim HY, 2021). Here we present an interim analysis of OS and safety in the full cohort.

Method: REFINE included patients with uHCC for whom a decision to treat with regorafenib was made by the treating physician prior to enrolment according to the local health authority approved label. The primary objective of this study is safety; secondary endpoints include OS, PFS, and treatment duration.

Results: Of the 1031 patients enrolled, 1008 were evaluable for analysis. Median age was 66 years (range 21–94); 83% had an ECOG PS of 0 or 1 (12% missing) at study entry; 32%/49%/4% had ALBI grade 1/2/3 (15% missing) at baseline; and 62%/12%/<1% were classified as Child–Pugh (CP) A/B/C (26% missing/not evaluable [NE]) at study entry. In total, 96% had prior sorafenib and 9% had ≥1 prior immunotherapy. In sorafenib tolerant patients (91%), 90% experienced a TEAE (most common: hand–foot skin reaction [32.5%]) and 51% experienced a grade ≥3 TEAE. In sorafenib intolerant patients (9%), 93% experienced a TEAE (most common: diarrhea [27%], hand–foot skin reaction [25%] and asthenia [19%]) and 60% experienced a grade ≥3 TEAE. Subgroup analyses of OS are shown (**Table**).

Conclusion: The real-world REFINE study supports the safety and effectiveness of regorafenib in a broader patient population with uHCC who had prior systemic treatment other than sorafenib, including immunotherapy, and included sorafenib intolerant patients. This interim analysis of OS favoured regorafenib when used early in the treatment sequence in patients with better liver function.

Figure:

	Regorafenib, % patients (N = 1008)	**Median OS, months (95% CI)**
Overall population	100	12.9 (11.4, 14.6)
CP grade at baseline		
A	62	15.2 (13.3, 16.2)
B	12	6.3 (4.9, 8.1)
Missing/NE	26	12.2 (9.4, 15.3)
ALBI grade at baseline		
1	32	19.8 (16.7, 24.6)
2	49	9.9 (8.5, 11.1)
Missing	15	12.4 (9.3, 15.3)
Prior immunotherapy	9	10.2 (7.4, 15.2)
Sorafenib intolerant	9	11.1 (8.6, 19.5)
Prior treatment lines*		
1 (sorafenib only)	82	13.8 (12.2, 15.3)
≥2	14	8.7 (7.4, 12.1)

*Patients with no prior treatment (1%) had a median OS of 6.7 months (95% CI 2.2, NE); patients with one prior treatment other than sorafenib (2%) had a median OS of 18.8 months (95% CI 6.8, NE).

OS-79
Circulating vesicles hold etiology-related protein biomarkers of cholangiocarcinoma risk, early diagnosis and prognosis mirroring tumour cells

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Background and aims: Cholangiocarcinomas (CCAs), heterogeneous biliary tumours with dismal prognosis, lack accurate early-diagnostic methods, especially important for individuals at high-risk (i.e. primary sclerosing cholangitis (PSC)). Here, we aimed to identify precise non-invasive CCA biomarkers.

Method: Serum extracellular vesicles (EVs) from patients with: i) isolated PSC (n=39); ii) PSC without clinical evidences of malignancy at sampling who developed CCA overtime (PSC to CCA; n=10); iii) concomitant PSC-CCA (n=14); iv) CCAs from non-PSC etiology (n=26); and v) healthy individuals (n=41) were analysed by mass-spectrometry. Diagnostic biomarkers of PSC-CCA, non-PSC CCA or CCAs regardless etiology (pan-CCAs) were defined, and their expression evaluated in human multi-organs and within CCA tumours at single-cell level. Prognostic EV-biomarkers for CCA were described.

Results: High-throughput proteomics identified candidate diagnostic biomarkers for PSC-CCA, non-PSC CCA or pan-CCA, independent to sex, age and CCA subtype. Machine learning logit modelling disclosed PLCH1/FGL1 algorithm with diagnostic value of AUC=0.903 and OR=27.8 for early-stage PSC-CCA vs isolated PSC, overpowering CA19-9 (AUC=0.608, OR=2.0). An algorithm combining SAMP/A1AT allowed the diagnosis of early-stage non-PSC CCAs compared to healthy individuals (AUC=0.863, OR=18.5). Noteworthy, the levels of 6 proteins (ALBU;FIBB;FLG1;IGHA1;TLN1;IMA8) showed predictive value for CCA development in patients with PSC before clinical evidences of malignancy. Multi-organ transcriptomic analysis revealed that serum EV-biomarkers were mostly expressed in hepatobiliary tissues and scRNA-seq analysis of CCA tumours indicated that some biomarkers –including PIGR,FGG,SERPINA1,FGL1– were mainly expressed in malignant cholangiocytes. Multivariable analysis revealed EV-prognostic biomarkers independent to clinical features, with FCN2/SDPR/FA9 panel being strongly associated to patients' survival.

Conclusion: Serum EVs contain etiology-specific protein biomarkers for the prediction, early diagnosis and prognosis estimation of CCA, representing a novel tumour cell-derived liquid biopsy for personalized medicine.

OS-186
Early Nivolumab addition to Regorafenib in patients with Hepatocellular Carcinoma progressing under 1st line therapy (GOING trial). Interim analysis and safety profile.

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Background and aims: Regorafenib (Rego) improves survival in patients with hepatocellular carcinoma (HCC) (RESORCE trial) while Nivolumab (Nivo) is also safe and active in terms of radiologic response (15-20% objective response) in second-line. The GOING trial (NCT04170556) is an investigator-initiated phase I/IIa study assessing the safety of Rego plus Nivo in HCC patients who progress and tolerate sorafenib (Cohort A) or who discontinue atezolizumab plus bevacizumab (Cohort B).

Method: Patients from cohort A receive Rego as monotherapy for the first 2 cycles (starting dose 160 mg/day, 3 weeks on/1 week off and adjusted for adverse events [AEs]) and Nivo is added at day 1 of cycle 3 (the first 10 patients at 3 mg/kg; since they did not present serious-AE (SAE), the final dose is 240 mg every two weeks). Treatment continues until unacceptable AEs, symptomatic tumour progression, patient decision or death. Safety is measured by the rate of AEs, rate of treatment related-AEs (Tr-AE), rate of AEs leading to treatment discontinuation and rate of death. Severity of AEs are evaluated according to CTCAE v.5.0. A futility analysis using the non-binding Lan & DeMets beta-spending functions with a boundary of p=0.814 is mandated when 32.8% of cohort A has data of tumour assessment at least at week 16 by RECIST 1.1.

Results: Fifty-one patients have been enrolled in cohort A as of May 15, 2021. The first 30 (BCLC-C 73%) were considered in this safety analysis. All patients developed at least one AE, 29 (96.7%) had Tr-AEs of any grade and 10 (33.3%) had grade 3 (no grades 4 or 5 have been reported). Ten (33.3%) patients had a Rego-related AE, 4 (13.3%) Nivo-related, and 4 (13.3%) Tr-AE grade 3 of special interest. Table 1 describes the profile Tr-AE occurring in > 10% of patients. Only 4 (13.3%) patients developed Tr-SAE, 3 (10 %) Rego-related and 3 (10 %) Nivo-related. Four patients discontinued the study due to physician decision, 2 for progression, and 2 for AEs (one related to study treatment). One patient had surgical resection after treatment discontinuation and a complete necrosis was observed at pathology.

Conclusion: The sequential combination of Rego-Nivo has a manageable safety profile. Less than one third of the patients developed grade 3/4 Tr-AE and there was no treatment-related death. Futility analysis allowed to continuing recruitment.

Figure: Table 1. Profile of Tr-AE occurring in > 10% in the first 30 included patients.

Adverse Events	Any Grade		Grade 3	
	Patients, n	Patients, %	Patients, n	Patients, %
Hand-foot-skin reaction	17	56.6	3	10.0
Asthenia	13	43.3	-	-
Diarrhea	11	36.7	1	3.3
Decreased Appetite	9	30.0	-	-
Arterial Hypertension	9	30.0	2	6.7
Hypertransaminasaemia	9	30.0	1	3.3
Hyperbilirubinaemia	6	20.0	1	3.3
Aspartate Aminotransferase Increased	6	20.0	1	3.3
Abdominal Pain	6	20.0	-	-
Dysphonia	5	16.7	-	-
Alanine Aminotransferase Increased	4	13.3	-	-

* None of these Tr-AE was grade 4- 5

POSTER ABSTRACT PRESENTATIONS

PO-4

Texture analysis using computed tomography data may be helpful for the risk stratification of clinical significant portal hypertension in patients with liver cirrhosis

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Texture analysis using computed tomography data may be helpful for the risk stratification of clinical significant portal hypertension in patients with liver cirrhosis

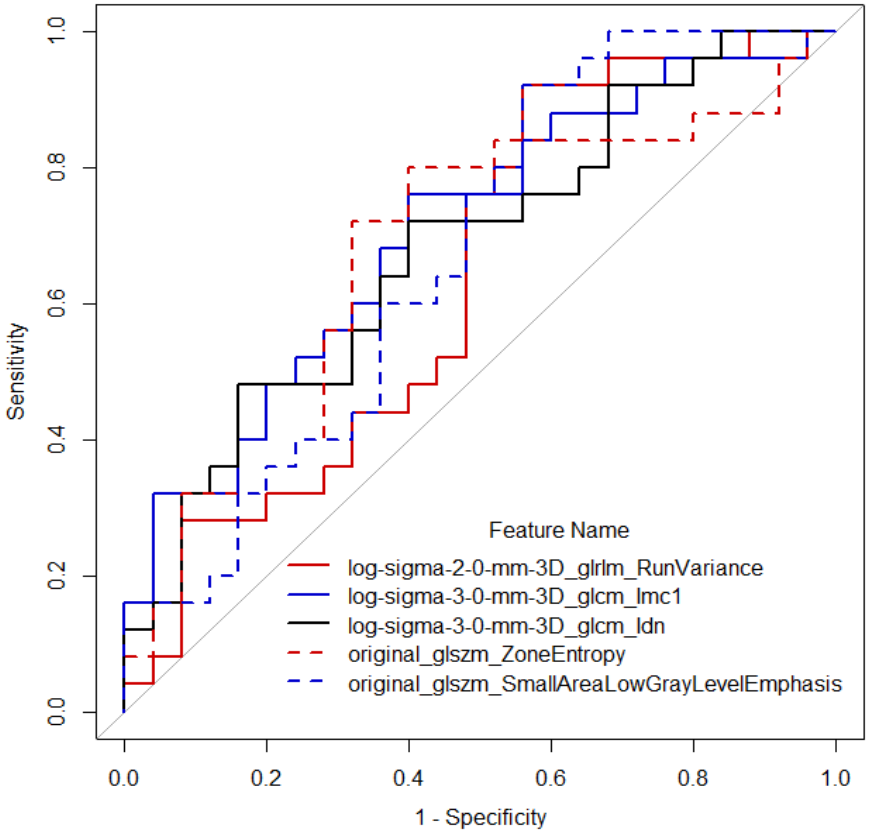
Background and Aims: Clinical significant portal hypertension (CSPH) in patients with liver cirrhosis is of great clinical significance and is defined as a hepatic venous pressure gradient (HVPG) > 10 mmHg, which can develop a series of complications. Current guidelines indicated that in patients with CSPH, a HVPG > 20 mmHg indicates high risk of failure to control variceal bleeding, early rebleeding, and high mortality. Thus, in this study, we aimed to stratify CSPH and identify the high-risk portal hypertension (HVPG > 20 mmHg) using the non-invasive method of texture analysis based on computed tomography (CT).

Methods: Sixty-one consecutive patients with liver cirrhosis between August 2020 and March 2021 were retrospectively enrolled in this study, all had both invasive HVPG measurements and contrast-enhanced CT within 14 days prior to the catheterization. Patients were divided into low-risk portal hypertension (HVPG ≤ 20 mmHg) and high-risk portal hypertension (HVPG > 20 mmHg). CT-based texture features of liver and spleen in the two groups were obtained by manual drawing of region of interest (ROI). Uni-variable logistic regression model was applied to select the significant features related to HVPG > 20 mmHg. Receiver operating characteristic (ROC) was used to test the diagnostic capacity of each feature.

Results: Of all the features, five texture features of liver (three features from Laplacian of Gaussian filter image and two features from Gray Level Size Zone) were identified statistically significant ($p < 0.05$), all of them have favorable ability to discriminate high-risk portal hypertension (HVPG > 20 mmHg, area under the curve (AUC), 0.638 - 0.704), of those, features of “log-sigma-3-0-mm-3D_glcm_Imc1” showed the best diagnostic performance (AUC = 0.704, $p = 0.012$), with a sensitivity of 76% and a specificity of 60%, an odds ratio (OR) of 2.18 (95% confidence interval (CI):1.12 - 4.23). However, no statistically significant feature of spleen was found ($p > 0.05$).

Conclusions: The texture features of liver based on CT may be used to predict high-risk portal hypertension (HVPG > 20 mmHg) and could serve as a potential predictive method to noninvasively stratify clinical significant portal hypertension.

Figure: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0.0
0.2
0.4
0.6
0.8
1.0
Feature Name
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log-sigma-3-0-mm-3D_glcm_Imc1
log-sigma-3-0-mm-3D_glcm_Idn
original_glszm_ZoneEntropy
original_glszm_SmallAreaLowGrayLevelEmphasis

PO-5
Quantitative parameters of esophageal varices based on computed tomography may be used for predicting severe varices in patients with liver cirrhosis

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Quantitative parameters of esophageal varices based on computed tomography may be used for predicting severe varices in patients with liver cirrhosis

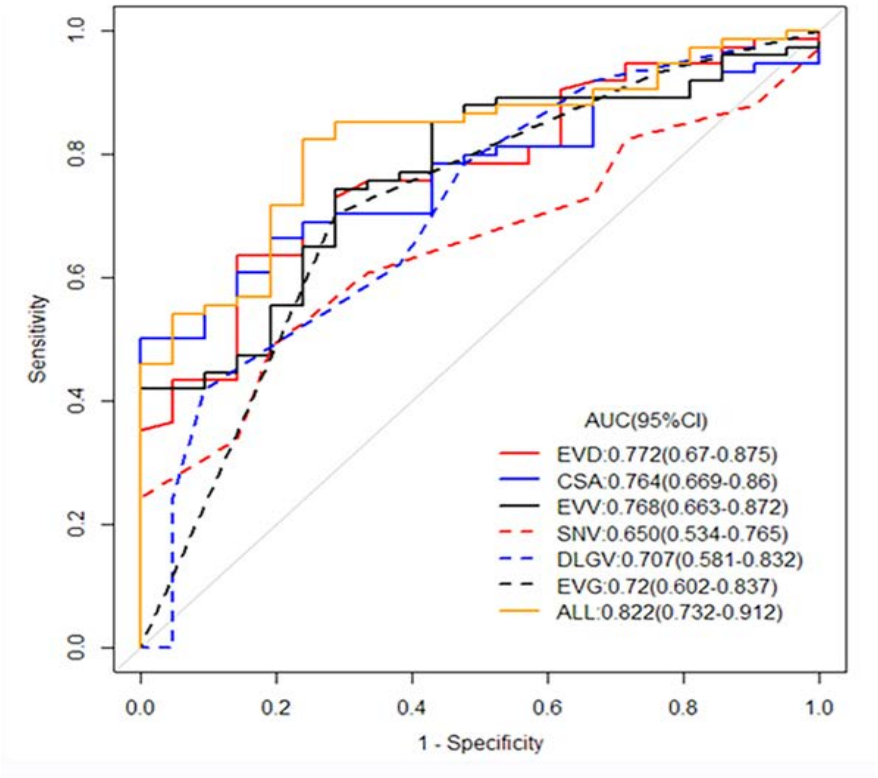
Background and Aims: Esophageal varices (EV) are the most common complication in patients with portal hypertension resulted from liver cirrhosis, and the subsequent variceal bleeding is the leading cause of death in those patients. The invasive procedure of endoscopy is now regarded as the reference standard for evaluation of EV, however, the utility of it is limited due to the invasive nature and the high-cost effectiveness. Thus, in this study, we aimed to assess whether the quantitative computed tomography (CT)-derived parameters can noninvasively predict the severity of EV and the risk of esophageal variceal bleeding (EVB).

Method: In this retrospective study, a total of 145 endoscopically confirmed EV patients were included and were divided into a conspicuous (mild-to-moderate EV, n = 39) and a non-conspicuous EV group (severe EV, n = 106), a bleeding (n = 89) and a non-bleeding group (n = 56). EV grade (EVG), EV diameter (EVD), cross-sectional surface area (CSA), EV volume (EVV), spleen volume (SV), splenic vein (SNV), portal vein (PV), diameter of left gastric vein (DLGV), and the opening type of LGV were measured independently using 3D-slicer. Univariate and multivariate logistic analysis were used to determine the independent factors and the receiver operating characteristic (ROC) curves were performed to evaluate the diagnostic performance.

Results: The difference of EVG, EVD, CSA, EVV, DLGV, SNV between the conspicuous and non-conspicuous EV group were statistically significant ($p < 0.05$), area under the curves (AUCs) of them for predicting severe EV were 0.72, 0.772, 0.704, 0.768, 0.707, 0.65, with corresponding sensitivities of 70.3%, 63.5%, 50%, 74.3%, 52.7%, 48.6%, specificities of 71.4%, 85.7%, 100%, 71.4%, 81%, 81% respectively. EVG, CSA (odds ratio (OR):3.258, 95% confidence interval (CI):1.597-6.647; OR:1.029, 95%CI:1.008 - 1.050) were found to be independent predictive factors. However, there was no significant difference of the included parameters between the bleeding and non-bleeding group ($p > 0.05$).

Conclusion: CT can be used as a non-invasive method to effectively predict the severity of esophageal varices, which may reduce the invasive screening of endoscopy and be used as a supplementary procedure in patients with portal hypertension.

Figure:


Sensitivity
1 - Specificity
0.0
0.2
0.4
0.6
0.8
1.0
AUC(95%CI)
EVD:0.772(0.67-0.875)
CSA:0.764(0.669-0.86)
EVV:0.768(0.663-0.872)
SNV:0.650(0.534-0.765)
DLGV:0.707(0.581-0.832)
EVG:0.72(0.602-0.837)
ALL:0.822(0.732-0.912)

PO-17
Mixed HCC-CCA originates from hepatic progenitor cells, is dependent on IL6 signaling and is ablated by senolytic agents

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Background and Aims: Primary liver cancer is the 3rd leading cause of cancer-related death worldwide. Primary liver cancers include: Hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (CCA) and Mixed HCC-CCA tumours. Chronic liver inflammation, which develops to cirrhosis, is a risk for the development of primary liver cancer. It has been suggested that hepatic progenitor cells (HPCs) could contribute to hepatocarcinogenesis. However, this has not yet been proven. HPCs proliferate in response to injury and chronic inflammation. In this study, we aimed to determine whether HPCs contribute to HCC, Mixed HCC-CCA or both types of tumours, in the MDR2 KO mouse model of inflammation-induced cancer.

Method: In order to enable tracing of progenitor cells, we generated a transgenic mouse based on the MDR2 KO that harbours a YFP reporter gene driven by the Foxl1 promoter. Foxl1 is expressed specifically in adult HPCs. These mice (MDR2 KOFoxl1CRE; RosaYFP) develop chronic inflammation by the age of 1 month and develop HCCs by the age of 14- 16 months, followed by mixed HCC-CCA tumours at the age of 18 months, as we have first observed, suggesting that the aged mice are a suitable model for mixed HCC-CCA tumours.

Results: In this model, we show that liver progenitor cells are the source of mixed HCC-CCA tumours, but they are not the source of HCC in the chronically inflamed liver. By generating mice with a Diphtheria toxin (DT) receptor in HPCs and administering DT, we ablated the progenitors, and we observed a significant reduction in the development of mixed HCC-CCA tumours but no change in HCCs. RNA-seq analysis revealed enrichment of the IL6 signaling pathway in mixed HCC-CCA tumours in comparison to HCC tumours. A single cell RNA-seq analysis revealed that in the liver, IL6 is expressed from both, immune cells and parenchymal cells which are in senescence, and that IL6 is part of the senescence-associated secretory phenotype (SASP). Upon administration of anti-IL6 antibodies to the MDR2 KOFoxl1CRE; RosaYFP mice, we inhibited the development of the mixed HCC-CCA tumours. Furthermore, by blocking IL6 transsignaling with sgp130, we also decreased mixed HCC-CCA tumours, indicating that mixed HCC-CCA tumours are dependent on IL6 transsignaling. The administration of a senolytic agent to these mice, also inhibited the development of mixed HCC-CCA tumours.

Conclusion: Our results suggest that mixed HCC-CCA but not HCC tumours, originate from HPCs in the inflammation induced liver cancer model, and that the driver of this process involves the IL6 signalling pathway that at least in part, derives from SASP of cells in senescence. These findings could enhance the development of new therapeutic approaches for mixed HCC-CCA liver cancer.

PO-22

Hepatocellular carcinoma is associated with an increased prevalence of metabolic syndrome after liver transplantation

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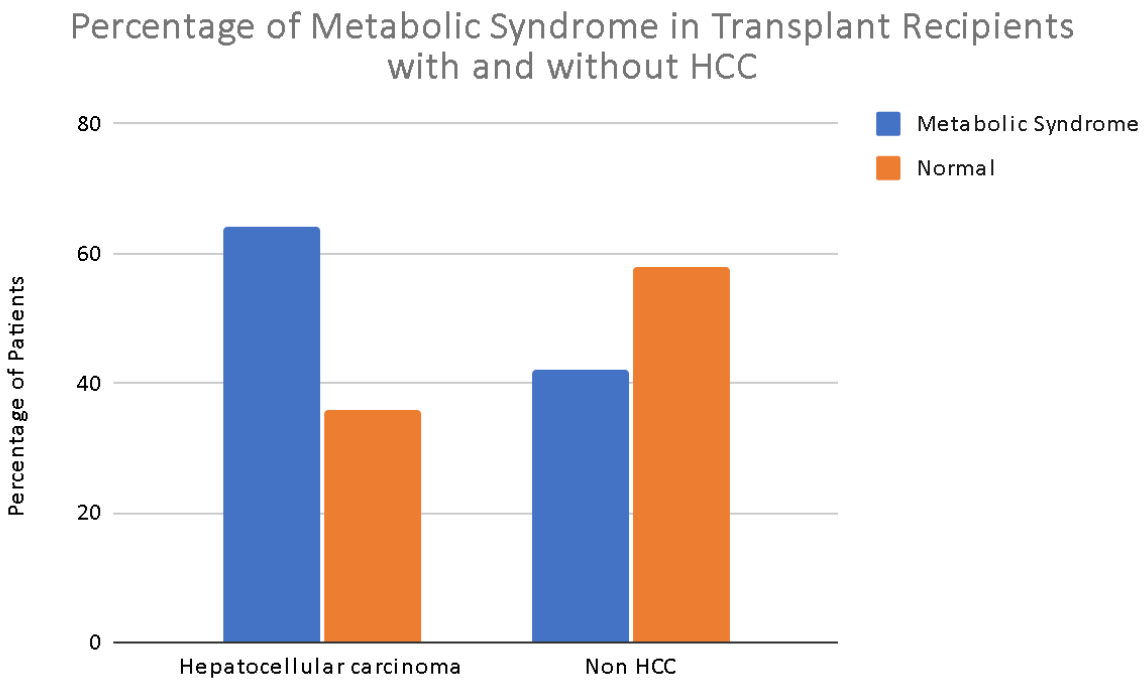
Background and Aims: Metabolic syndrome (PTMS) is common in patients after liver transplantation. PTMS is associated with an increase in post-transplant morbidity including cardiovascular events and kidney failure. Patients with a history of other malignancies are at higher risk of metabolic syndrome. The objective of this study was to determine if patients with hepatocellular carcinoma (HCC) are at greater risk for PTMS.

Method: This is a retrospective study of 143 consecutive patients who underwent a liver transplant from July 1, 2016 to December 31, 2017 at a single large tertiary care liver transplant centre. Patient charts were reviewed for demographics, immunosuppression, and metabolic syndrome at one year based on the International Diabetes Foundation Criteria. The primary outcome was the presence of metabolic syndrome one year after liver transplant. Factors associated with the presence of PTMS were analysed. This study was approved by the Institutional Review Board.

Results: The leading indication for transplant in this group was alcoholic liver disease followed by hepatitis C and non-alcoholic steatohepatitis. HCC was an indication for transplant in 24%(n=36). The prevalence of PTMS one year after transplant was 47% (n=67). In the univariate analysis, older patients, pre-transplant diabetes mellitus, NASH and HCC as indications for liver transplant were associated with the presence of PTMS at 1 year. Utilizing these factors, a multivariate model was created. This demonstrated that pre-transplant diabetes (OR: 4.10, CI: 1.69-9.92, $p<0.002$) and HCC (OR: 2.66, CI: 1.08-6.53, $p<0.03$) were independently associated with an increased prevalence of PTMS at 1 year. PTMS was found in 64% of patients with HCC compared to 42% of patients without HCC ($p<0.02$) (Figure 1). Moreover, transplanted HCC patients with metabolic syndrome had an average of 2.75 criteria associated with metabolic syndrome compared with 2.19 (p-value 0.02) in non-HCC patients.

Conclusion: Presence of HCC at time of transplant was independently associated with an increase in the prevalence of PTMS one year after transplant. Implementation of measures to reduce the risk of the metabolic syndrome with immunosuppression modification, lifestyle management, and medication management is recommended in liver transplant recipients with a history of HCC.

Figure:


Percentage of Metabolic Syndrome in Transplant Recipients with and without HCC
Metabolic Syndrome
Normal
Percentage of Patients
80
60
40
20
0
Hepatocellular carcinoma
Non HCC

PO-24

Occult hepatitis B virus infection and hepatocellular carcinoma occurrence after hepatitis C virus eradication: the silent enemy

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Background and Aims: Occult hepatitis B virus infection (OBI) has been reported to have a negative impact on the progression of liver cirrhosis and the development of hepatocellular carcinoma (HCC). Moreover, current evidence suggests a possible reactivation of HVB, lower response to antiviral treatment, and higher rates of HCC in patients with OBI and chronic hepatitis C virus infection (HCV). Our primary objective was to assess the risk factors associated with HCC development after direct acting antiviral (DAA) therapy in patients with chronic HCV infection. As a second objective, we aimed to determine the prevalence of seropositive OBI and to evaluate its impact on HCC occurrence.

Method: A total of 992 consecutive patients with chronic HCV infection that were treated with DAAs during 2015–2020 in the Institute of Gastroenterology and Hepatology Iasi, were followed-up by clinical visits with blood sampling, ultrasound±computed tomography and liver elastography until March 31, 2021.

Results: The study group included 752 (75.8%) patients with liver cirrhosis, 198 (20%) with significant to severe fibrosis and 42 (4.2%) with minimal or no fibrosis. The prevalence of anti-HBc seropositivity was 4.6%, out of which 2 (4%) patients had positive anti-HBs and 13 (28%) had detectable DNA HBV ranging between 49-200 IU/mL. Overall, 5 (0.5%) patients were non-responders to DAA therapy; all patients with OBI obtained sustained virological response (SVR). Throughout the follow-up period (mean 60.11±3.87 months), 59 (5.9%) of the 992 patients developed HCC, mainly females (55.9%), with a mean age of 68±9.6 years. Pearson's chi-square analysis of predictive factors for HCC included OBI (odds ratio [OR]/ 95% confidence interval [CI]: 2.519/1.022-6.206), diabetes mellitus (HR/CI: 4.256/2.389-7.584), age (HR/CI: 6.650/3.541-12.489), dysplastic nodules (HR/CI: 27.719/13.076-58.761) and male sex (HR/CI: 1,730 /1.016-2.946).

Conclusion: In addition to the classic risk factors for HCC after DAAs in chronically HCV-infected patients such as older age, male sex, diabetes, and the presence of dysplastic nodules, we found that OBI appears to have a long-term negative impact on hepatocarcinogenesis after obtaining SVR. This requires further research in order to establish the mechanism through which OBI promotes HCC and to evaluate the possibility of adopting HBV sterilizing strategies such as direct-targeting cccDNA agents in patients with HCV eradication and OBI.

PO-25

A new 3D Slicer plug-in for the interactive annotation and segmentation of liver anatomy

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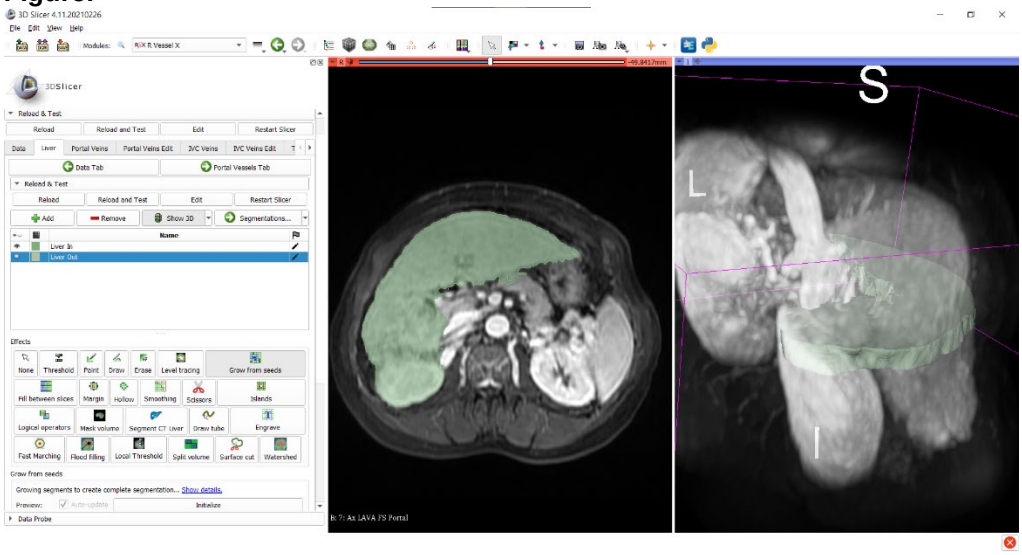
Background and Aims: Annotation plays a key role in the creation of reference datasets that are useful to evaluate medical image processing algorithms and to train machine learning based architectures. RVXLiverSegmentation is a 3D Slicer plug-in aiming at speeding-up the interactive annotation and segmentation of liver anatomy from medical images (CT or MRI for instance).

Method: The RVXLiverSegmentation provides 7 main tabs: loading and managing medical imaging data; liver segmentation; annotation of portal veins and segmentation; editing portal veins segmentation; annotation of inferior vena cava and segmentation; editing inferior vena cava segmentation; tumour segmentation. Once the medical image data is loaded into 3D Slicer, the liver can be segmented either by using interactive tools (e.g. region growing) or by an automatic deep learning-based algorithm (for CT scans only). Then, the reconstructions of hepatic vessels are based on tree structures interactively built by the user, who places the nodes of important branches and bifurcations (with specific anatomical nomenclature) into the scene of the medical image to be processed. After this step, a VMTK (Vascular Modeling Tool Kit) module segments the vessels by using those graphs as initialization patterns; also, the user can edit this segmentation. The last tab allows the user to segment interactively possible tumours with dedicated tools.

Results: We compared the time of segmentations obtained by RVXLiverSegmentation and by embedded image processing General Electric AW solution (Server 3.2). We measured times with a cohort of 6 "healthy" patients (i.e. not suffering from any hepatic disease, G1) and 4 patients with liver cancer and cirrhosis (G2). We have obtained a significant speed-up in the segmentation of liver volume and inner vessels in favor of our plug-in, of 8.0±3.0 for G1 and 4.5±4.2 for G2.

Conclusion: RVXLiverSegmentation is a promising tool for the creation of annotated datasets, and the faithful 3D reconstruction of liver anatomy from medical images. We first would like to integrate advanced deep learning models for liver and hepatic vessels segmentation into our RVXLiverSegmentation plug-in, in order to provide automatic reconstructions that can be then edited by the user. Another important work concerns the VMTK module, which needs more adaptations for MRI processing. Finally, a more complete evaluation protocol will be conducted by considering larger patient cohorts.

Figure:



PO-28

Mathematical modeling of cancer cells and vasculature dynamics with serological and imaging biomarkers suggests synergistic effects of TACE and TKIs in HCC patients

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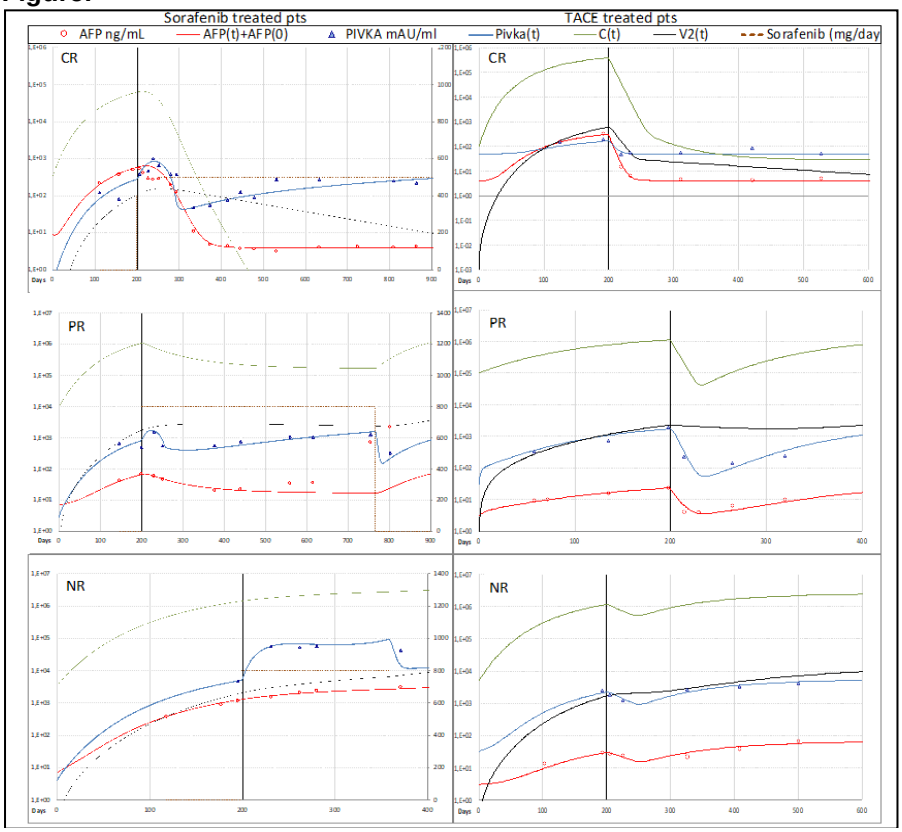
Background and Aims: Transarterial-chemoembolization (TACE) and tyrosine-kinase inhibitors (TKI) represent first-line treatments in intermediate and advanced hepatocellular carcinoma (HCC). Deepening TKI and TACE response mechanisms may provide insights to optimize their combination. We developed a physic-mathematical model to analyse cancer cells and tumour vasculature dynamics in HCC patients (pts) using serum biomarkers combined with tumour digital imaging before and after TACE or TKI treatment.

Method: Ten pts (F/M: 2/8, median age: 65y, stage: 1 BCLC-B and 9 BCLC-C) who received TKIs (1 regorafenib, 9 sorafenib) and 8 pts (F/M: 5/3, median age: 77y, all BCLC-B) who underwent TACE (doxorubicin+DC-beads), with serological and imaging data suitable for modelling analysis, were enrolled. Circulating HCC biomarkers (alpha-fetoprotein, AFP and protein induced by vitamin K absence-II, PIVKA-II) were measured by commercial assays (Abbott, Fujirebio). HCC volume and densitometry were measured by CT scans (GE Advantage Workstation 4.6). The model used for fitting experimental data is described at https://doi.org/10.3390/cancers13092064

Results: The model was able to fit AFP and PIVKA-II measures independently on therapy response in both TKI [4 Complete Response (CR), 4 Partial Response (PR), 2 Progressive Disease (PD)] and TACE [2 CR, 5 PR, 1 PD] treated pts (Figure). The computed anti-angiogenetic and anti-proliferative effectiveness of TKIs were 13.7 and 7.0-fold higher as compared to TACE, by contrast TACE reached the maximal therapeutic effect in <1 day, and TKIs between 4.6-99.9 days. AFP decline after TACE followed its natural decay (0.10-0.16 day-1), whereas it occurred with delay in TKIs responders. An early spike of PIVKA-II levels was observed in most pts receiving TKIs, but not after TACE, suggesting that PIVKA-II production rate can increase only when the ischemia onset is slow. Anti-angiogenesis effectiveness was, on average, 8-fold higher in CR that in NON-CR pts, regardless to the type of treatment. CR pts showed an accelerated vasculature decay also, which was maintained in TKIs treated pts despite significant lowering of the doses.

Conclusion: Cancer cells and tumour vasculature dynamics in HCC patients can be analysed by modeling the kinetics of serum biomarkers combined with tumour digital imaging. Our model supports potential synergic effects of TKIs and TACE, although further studies are required to define the best strategy of combination.

Figure:


Sorafenib treated pts
TACE treated pts
AFP ng/mL
AFP(t)+AFP(0)
PIVKA mAU/ml
Pivka(t)
C(t)
V2(t)
Sorafenib (mg/day
CR
CR
PR
PR
NR
NR

PO-29

Independent validation of a glycomics-based test associated with risk of HCC development in cirrhosis: a new tool for screening optimisation?

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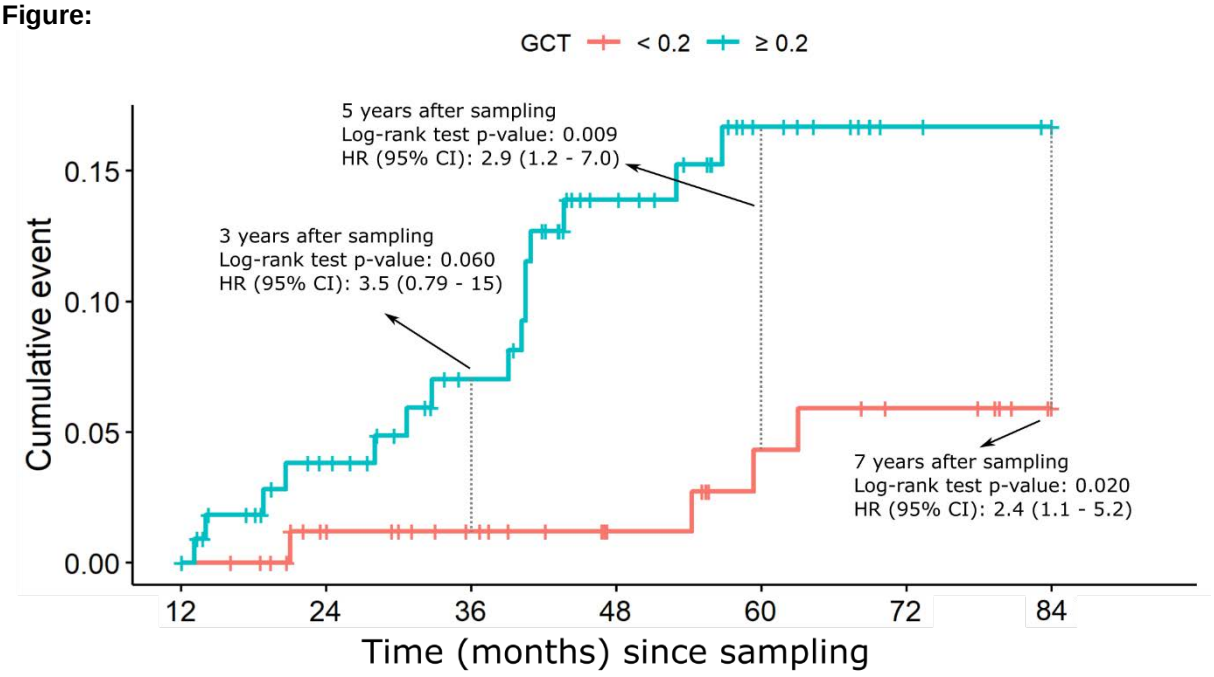
Background and Aims: Cirrhosis is the main risk factor for the development of hepatocellular carcinoma (HCC). Six-monthly screening with ultrasound is advocated for the surveillance of cirrhotic patients. We recently showed that a glycomics-based test (GlycoCirrhoTest [GCT]) can provide additional information regarding the risk of HCC development in cirrhotic patients.
The aim of this study is to provide an independent clinical validation of the GCT for the assessment of the risk of HCC development in cirrhosis.

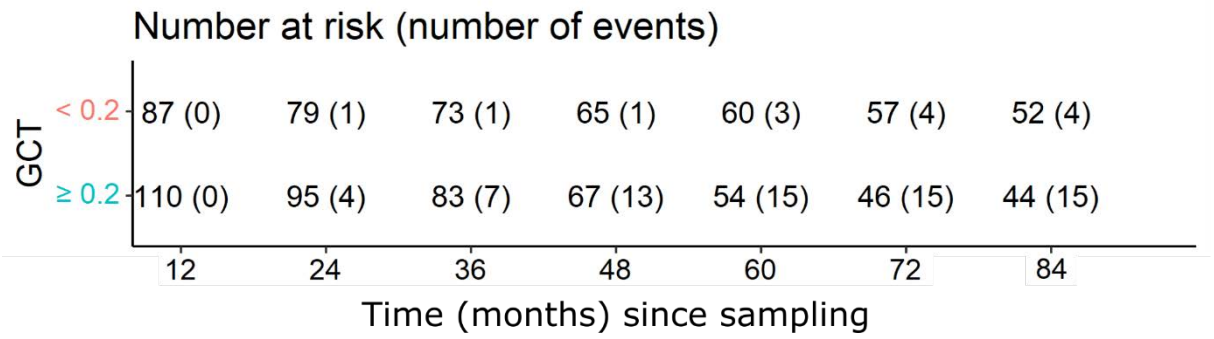
Method: Validation study on serum samples of patients with established compensated cirrhosis (CHILD Pugh A&B) in a tertiary liver centre. Serum N-glycan profiling was performed and GCT was calculated at baseline using DNA sequencer assisted fluorophore assisted capillary electrophoresis. During the follow up period, patients were screened for the presence of HCC every 6 months with ultrasound and alpha foeto protein (AFP) measurements.

Results: A total of 198 cirrhotic patients were followed during a median follow up time of 7 years. Twenty-nine patients developed HCC and one died during follow up. At baseline, the mean GCT value was significantly higher in patients who developed HCC within 3 and 5 years compared to patients who did not develop HCC (Welch's t-test, p-value 3 years: 0.034, 5 years: 0.022). Hazard ratio for HCC development at 5 years based on GCT was 2.9 (95% CI, 1.2 – 7.0). Applying the same cut-off as from the proof-of-concept study (0.2), the negative predictive value of GCT for HCC development was 98.9%. Figure 1 illustrates the discriminative power of this biomarker in patients with cirrhosis. GCT is based on changes in serum protein glycosylation related to cirrhosis nodularity and malignant transformation.

Conclusion: This independent validation study confirms that GCT is a glycomics-based test that provides additional information for risk assessment of HCC development in cirrhosis. This information could be used to develop personalised HCC screening programs in cirrhotic patients according to the value of GCT. Moreover, refocusing of the screening resources to the reduced number of cirrhosis patients who truly are at elevated risk for developing HCC may result in earlier detection of more HCC cases, for instance by making contrast-enhanced MRI screening cost-effective.

Figure:


GCT < 0.2 ≥ 0.2
5 years after sampling
Log-rank test p-value: 0.009
HR (95% CI): 2.9 (1.2 - 7.0)
3 years after sampling
Log-rank test p-value: 0.060
HR (95% CI): 3.5 (0.79 - 15)
7 years after sampling
Log-rank test p-value: 0.020
HR (95% CI): 2.4 (1.1 - 5.2)
Cumulative event
0.15
0.10
0.05
0.00
12
24
36
48
60
72
84
Time (months) since sampling


Number at risk (number of events)
GCT
< 0.2
≥ 0.2
87 (0)
79 (1)
73 (1)
65 (1)
60 (3)
57 (4)
52 (4)
110 (0)
95 (4)
83 (7)
67 (13)
54 (15)
46 (15)
44 (15)
12
24
36
48
60
72
84
Time (months) since sampling

PO-30

Pretransplant changes in serum protein glycosylation are associated to risk of HCC recurrence after liver transplantation and provide a potential prognostic biomarker: a proof-of-concept study

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Background and Aims: Hepatocellular carcinoma (HCC) recurs after liver transplantation (LT) in 10% of patients. Changes in protein glycosylation have been described during the development of HCC. Study goal was to assess the risk of HCC recurrence after LT, according to changes in serum protein glycosylation before LT.

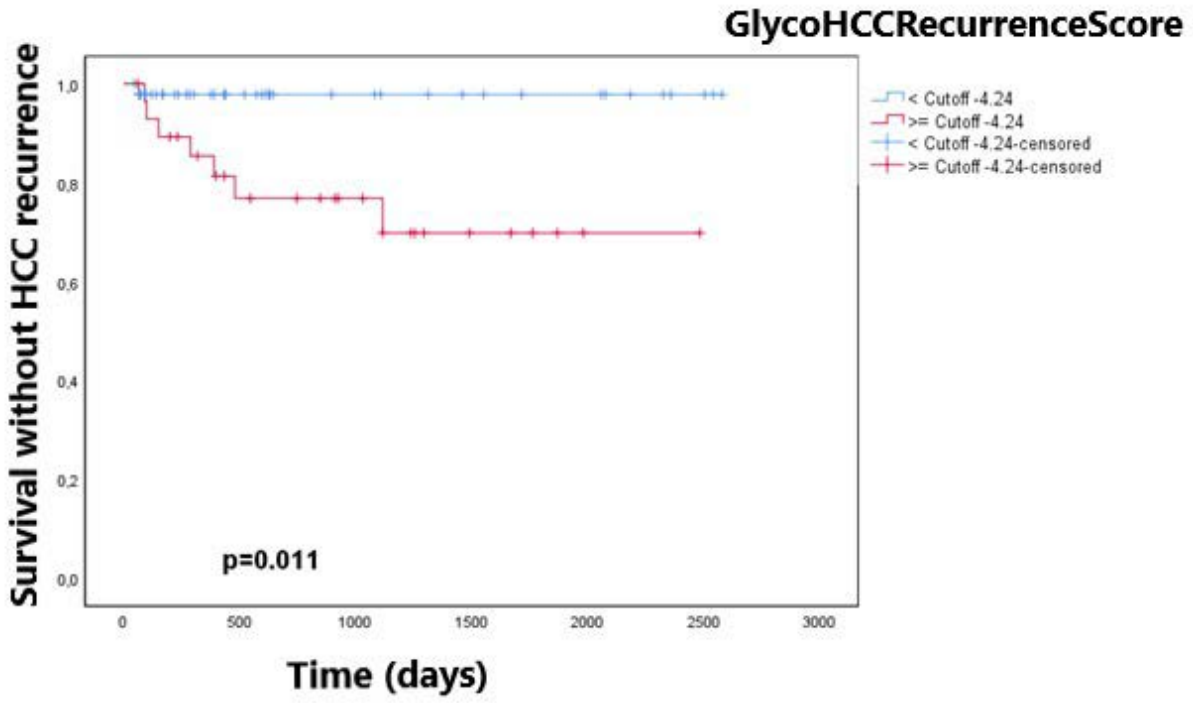
Method:
A prospective study was performed in patients receiving LT between July 2011 and September 2018. A whole serum protein N-glycan profile was assessed using DNA sequencer assisted fluorophore assisted capillary electrophoresis, using a validated high-throughput protocol. For every sample, 13 glycans were quantified. Patients were followed until HCC recurrence or death. Specific changes in serum protein glycosylation profiles were analysed in patients with HCC recurrence compared to patients without.

Results:
Amongst 225 consecutive liver transplant patients, 76 patients suffered from HCC before LT. Main indications were related to alcoholic cirrhosis (47.4%), HCV infection (21.1%) and NASH (15.8%). Eight patients (10.5%) developed HCC recurrence after a median follow up time of 9.5 months after LT. Seventy-four patients (97%) fulfilled Milan criteria.

Significant differences in the relative abundance of 5 serum glycans were present in patients with HCC recurrence compared to patients without (Cox regression analysis). Based on these changes, a composite biomarker was developed (GlycoHCCRecurrenceScore). This score integrates an increased presence of triantennary glycans with and without branch and core fucosylation (NA3, NA3Fc and NA3Fbc) and a decreased presence of undergalactosylated glycans NGA2F and NGA2FB in patients with HCC recurrence. This biomarker panel shows an AUC of 0.855 (p=0.001; 95% CI 0.731-0.979) for association with HCC recurrence. Using an optimized cut-off (-4.24), sensitivity fwas 87.5% and specificity 67.6%. Only 2.1% of patients with a value below this cut-off showed HCC recurrence, compared to 24.1% of patients with values above this cut-off (p=0.011). PPV was 72.98% and NPV 84.39%. Figure 1 illustrates the discriminative value of this biomarker. In a univariate cox regression analysis other factors related to HCC recurrence in this cohort were diameter of the largest lesion before LT and the presence of perineural or lymphovascular invasion in the explant liver. In a multivariate analysis, the biomarker showed an independent relation with HCC recurrence (HR 1.931; p=0.008:1.184-3.149).

Conclusion: A glycomics based serum biomarker panel is strongly associated with tumour recurrence in a cohort of LT patients with HCC, even if adhering to Milan criteria. In a multivariate analysis, this biomarker was the only pretransplant discriminative parameter of HCC recurrence in this cohort. The biomarker could potentially increase the prediction of HCC recurrence and improve allocation strategies in LT candidates with HCC.

Figure:


GlycoHCCRecurrenceScore
Survival without HCC recurrence
1,0
0,8
0,6
0,4
0,2
0,0
p=0.011
0
500
1000
1500
2000
2500
3000
Time (days)
< Cutoff -4.24
>= Cutoff -4.24
< Cutoff -4.24-censored
>= Cutoff -4.24-censored

PO-38
Circulating tumour DNA methylation markers for diagnosis of liver cancer: A multicentre diagnostic trial

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Background and Aims: CtDNA has been considered a high diagnostic accuracy for detection of early-stage liver cancer, however, the efficacy for the detection of liver cancer has not yet been identified in an independent cohort.

Method: The patients aged 35-70 years who were diagnosed with liver cirrhosis histologically, moderate and severe fatty liver, AFP (alpha-protein, AFP) ≥20 ng/ml, or having a positive ultrasound characteristic were eligible for this study. Traditional screening methods (AFP combined with ultrasound) and ctDNA methylation liquid biopsy were performed on all participants. Enhanced CT or enhanced MRI were further offered for confirmation targeted for suspicious cases from traditional screening methods or ctDNA methylation liquid biopsy. The target data set is divided into a training set and test set according to the ratio of 7:3. The logistics region model with the ridge generalization method was used to develop the model in the training set and then evaluated in the test set. The area under receiver operating characteristic curve (AUROC) was used to assess the performance of the model

Results: A total of 1233 individuals were recruited for our study, and 45 cases were diagnosed with liver cancer. The sensitivity of ultrasound, AFP, ultrasound combined with AFP, and the combination of the three methods (Overall) were 51.11%, 40.00%, 71.11%, and 67.07%, respectively. The specificity was 98.74%, 96.63%, 95.18% and 95.20%, respectively. In the training set, the sensitivity of ultrasound, AFP, ultrasound combined with AFP, and overall is 51.46%, 40.39%, 70.80%, and 68.19% respectively. The specificity was 98.74%, 96.58%, 95.37% and 95.38%, respectively. The AUCs of AFP, ultrasound and ctDNA methylation were 0.74, 0.91, and 0.73 respectively. The AUCs of ctDNA methylation combined with AFP or ctDNA methylation combined with ultrasound were 0.83 and 0.86 respectively. The AUCs of AFP combined with ultrasound or ctDNA methylation combined with the mixture of ultrasound and AFP were 0.89 and 0.92 respectively. In the test set, the sensitivity of ultrasound, AFP, ultrasound combined with AFP, and overall was 50.28%, 39.15%, 71.74%, and 65.19% respectively. The specificity was 98.74%, 96.65%, 95.24% and 95.24%, respectively. The AUCs of AFP, ultrasound and ctDNA methylation were 0.74, 0.74, and 0.73 respectively. The AUCs of ctDNA methylation combined with AFP or ctDNA methylation combined with ultrasound were 0.81 and 0.85 respectively. The AUCs of AFP combined with ultrasound or ctDNA methylation combined with the mixture of ultrasound and AFP

Conclusion: Our findings support that ctDNA methylation can improve the screening efficiency of liver cancer.

Figure:

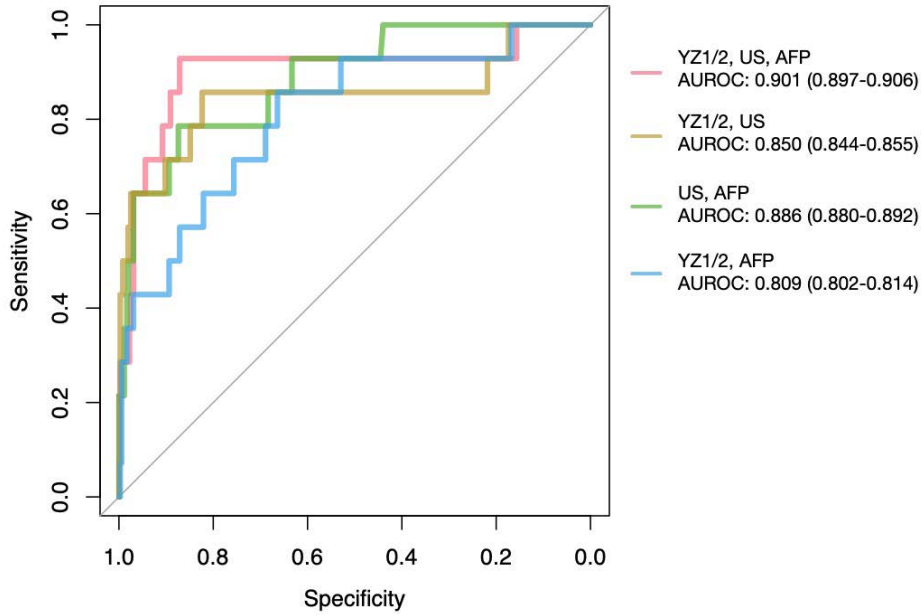

Figure 1 ROC curve for the detection of liver cancer (The combination of YZ1/2, AFP and Ultrasound has the best performance, YZ1/2 referring to the methylation markers)

PO-47

The clinical experience with stereotactic ablative radiotherapy for hepatocellular carcinoma in a tertiary referral centre: a case series

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Background and Aims: To assess safety and efficacy for stereotactic ablative radiotherapy (SABR) in patients with hepatocellular carcinoma (HCC) in a western European setting, as well as motives for its use.

Method: We retrospectively analysed 18 patients with HCC confined to the liver, treated with SABR (2008-2020). Primary outcome was radiological local control (LC), defined as complete response, partial response (≥30% reduction) or stable disease using mRECIST. Secondary outcomes were 1- and 2-year overall survival (OS), histopathologic assessments of resected livers and adverse events.

Results: 18 patients were included, median age 66 (range 46 - 88) with cirrhosis in 15 (13 Child Pugh (CP)A; 2 CPB). Motives for SABR were: other therapies not possible or feasible (n = 12, mostly due to tumour localization); comorbidities (n = 3); failed selective internal radiotherapy (n = 2) and patient preference for SABR (n = 1). No clinically significant adverse events were reported. Median follow-up was 10 months (IQR 7 - 24). Initial LC (initial imaging 3-5 months after SABR) was 100% (15/15, 3 not evaluable (NE); 1- and 2-year LC were 85.7% (6/7) and 100% (4/4). Median survival at analysis was 14 months (IQR 7 - 29). 1- and 2-year OS were 73.3% and 42.9%. 5 patients underwent liver transplantation after SABR. Histopathologic assessment of the tumour (available in 4) showed a scar with central necrosis, along with vital tumour tissue around the original tumour site in all.

Conclusion: SABR resulted in satisfactory radiological local tumour control, with low toxicity in this small heterogeneous group and was feasible as a bridge to transplantation in patients with HCC confined to the liver.

Figure:

Patient	Barcelona-Clinic Liver- Cancer stage	Longest diameter (mm)	Biologically effective dose 10	Initial Local Control (LC)	1-year LC	2-year LC
1	A	16	132	PR	CR	NE, death due to LF
2	A	24	132	NE, unquantifiable	NE, OLT	-
3	A	29	72	SD	SD	NE, death due to LF
4	B	43	86	CR	NE, OLT	-
5	A	29	103	SD	NE, additional TACE	-
6	0	20	86	CR	CR	CR
7	0	16	79	NE, unrelated death	-	-

8	A	24	103	CR	NE, OLT	-
9	B	42	103	CR	NE, death due to LF	-
10	A	18	151	NE, lost to FU	-	-
11	B	48	72	SD	PR	PR
12	A	24	151	CR	CR	PR
13	B	86	60	PR	PR	CR
14	B	37	100	PR	NE, lost to FU	-
15	0	15	113	PR	NE, unrelated death	-
16	B	49	60	SD	NE, OLT	-
17	A	30	66	PR	NE, OLT	-
18	0	16	100	PR	PD	NE, SABR <2 years

CR: complete response; PR: partial response, SD: stable disease; PD: progressive disease; NE: non-evaluable; LF: liver failure; OLT: orthotopic liver transplantation; TACE: transarterial chemoembolization; FU: follow-up.

PO-58

The systemic inflammatory response identifies patients with adverse clinical outcome from immunotherapy in hepatocellular carcinoma.

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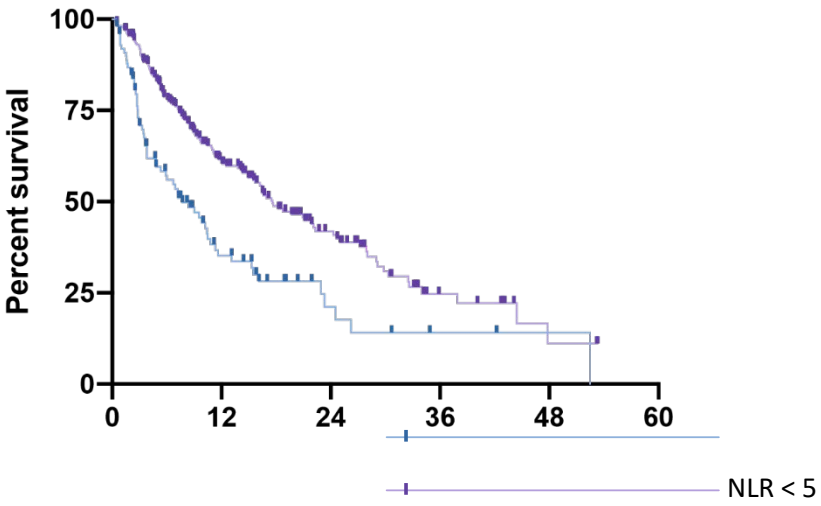
Background and Aims: Systemic inflammation is a hallmark of cancer, and it has a pivotal role in HCC development and progression. The role of systemic inflammation in influencing outcomes of patients treated with immunotherapy for HCC has not been fully elucidated.

Method: We conducted a retrospective study including 362 patients receiving immune-checkpoint inhibitors (ICIs) across 3 continents, evaluating the influence of neutrophiles to lymphocytes ratio (NLR), platelet to lymphocytes ratio (PLR) and prognostic nutritional index (PNI) on overall (OS), progression free survival (PFS) and radiologic responses.

Results: In our 362 patients treated with immunotherapy, median OS and PFS were 9 and 3.5 months respectively. Amongst tested inflammatory biomarkers, patients with NLR ≥ 5 had shorter OS (7.7 vs 17.6 months, $p < 0.0001$), PFS (2.1 vs 3.8 months, $p = 0.025$) and lower ORR (12% vs 22%, $p = 0.034$), similarly, patients with PLR ≥ 300 reported shorter OS (6.4 vs 16.5 months, $p < 0.0001$) and PFS (1.8 vs 3.7 months, $p = 0.0006$). NLR emerged as independent prognostic factors for OS in univariate and multivariate analysis (HR 1.95, 95%CI 1.45-2.64, $p < 0.001$; HR 1.73, 95%CI 1.23-2.42, $p = 0.002$) and PLR remained an independent prognostic factor for both OS and PFS in multivariate analysis (HR 1.60, 95%CI 1.6-2.40, $p = 0.020$; HR 1.99, 95%CI 1.11-3.49, $p = 0.021$).

Conclusion: Systemic inflammation measured by NLR and PLR is an independent negative prognostic factor in HCC patients undergoing ICI therapy. Further studies are required to understand the biological mechanisms underlying this association and to investigate the predictive significance of circulating inflammatory biomarkers in HCC patients treated with ICIs.

Figure: OS by pre-treatment NLR



PO-64
Gut bacteria modulate anti-tumour immunity in patients with Hepatocellular Carcinoma

Dhruti Devshi[1 2], Sandra Phillips[1 2], Helen Edwards[1 2], Nicola Harris[1 2], Melissa Preziosi[3], Abid Suddle[3], Antonio Riva[1 2], Shilpa Chokshi[1 2]

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Background and Aims: The gut microbiota and their metabolites play a key role in the response to checkpoint receptor (CR) inhibitors. Interestingly, antibiotics can also limit cancer progression by modulating the gut microbiota and their metabolites. The role of bacteria and their metabolites in modulating anti-tumour immunity in hepatocellular carcinoma (HCC) is not understood and is the focus of this study.

Method: Peripheral blood mononuclear cells (PBMCs) from HCC patients were stimulated in vitro with a peptide pool spanning seven HCC tumour-associated antigens with/without priming with formaldehyde-fixed Escherichia coli DH5a (EC, 50 bacteria-per-cell) or EC supernatants (20% v/v, representing bacterial metabolites). After 3 days, the expression of inhibitory immune checkpoints and functional markers (including PD-1, PD-L1, Granzyme-B, Perforin, IL-10 and IFNg) were assessed on CD4, CD8 and MAIT T cells by flow cytometry. Thirty-four secreted cytokines (including IL-6, IL-23, IL-2, IL-1a, IL-10, IL-18 and IFNg) were measured in PBMC culture supernatants by Luminex. For the analysis, peptide-only PBMC cultures were compared with EC-cell-primed or EC-supernatant-primed peptide-stimulated PBMC cultures.

Results: Immunosuppressive PD-1, PD-L1 and IL-10 expression was significantly higher in bacteria-primed HCC-specific CD4+ T-cells (p=0.024, 0.0061 and 0.037 respectively) compared to HCC peptide-stimulated only PBMCs. A significant increase in pro- and anti-inflammatory cytokines (including IL-6, IL-23, IL-2, IL-1a, IL-18, IL-10 and IFN gamma) was observed in bacteria-primed HCC peptide-stimulated PBMC cultures compared to HCC peptide-only stimulated PBMCs (p=0.037, 0.037, 0.0098, 0.0061, 0.0061, 0.0061, respectively). No significant differences were seen in MAIT cells, or granzyme-B/perforin expression between HCC peptide-stimulated only and bacteria-primed HCC peptide-stimulated PBMC cultures.

Conclusion: These results reveal that bacterial priming induces inhibitory checkpoint receptor expression on anti-tumour T-cells and promotes an immunosuppressive inflammatory landscape in HCC. Further studies investigating bacterial modulation of anti-tumour immunity in HCC are warranted.

PO-68
Hepatocellular carcinoma alters granulopoiesis to produce neutrophils with an immature phenotype

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Email: daniel.geh@newcastle.ac.uk

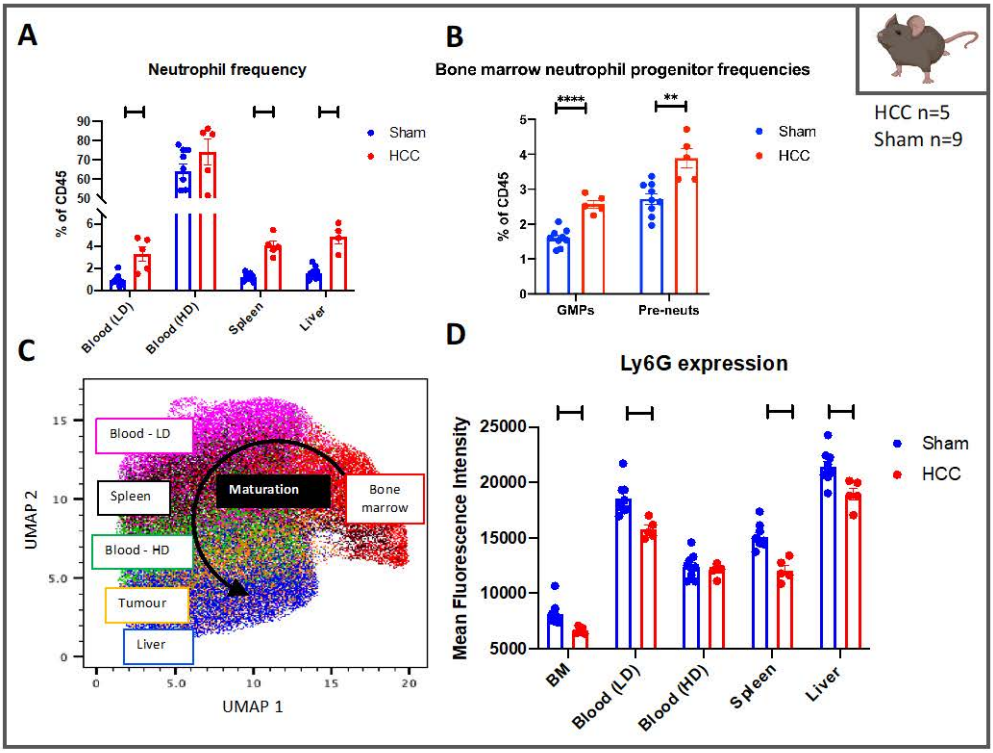
Background and Aims: Despite immunotherapy being the first line treatment for advanced hepatocellular carcinoma (HCC), the majority of HCCs are resistant, especially in the context of non-alcoholic fatty liver disease (NAFLD). Neutrophils have emerged as important drivers of HCC, exerting pro-tumour functions such as creating an immunosuppressive tumour microenvironment. Despite this little is known about how HCC influences neutrophils. Here we aim to characterize the effect HCC has on neutrophil phenotype.

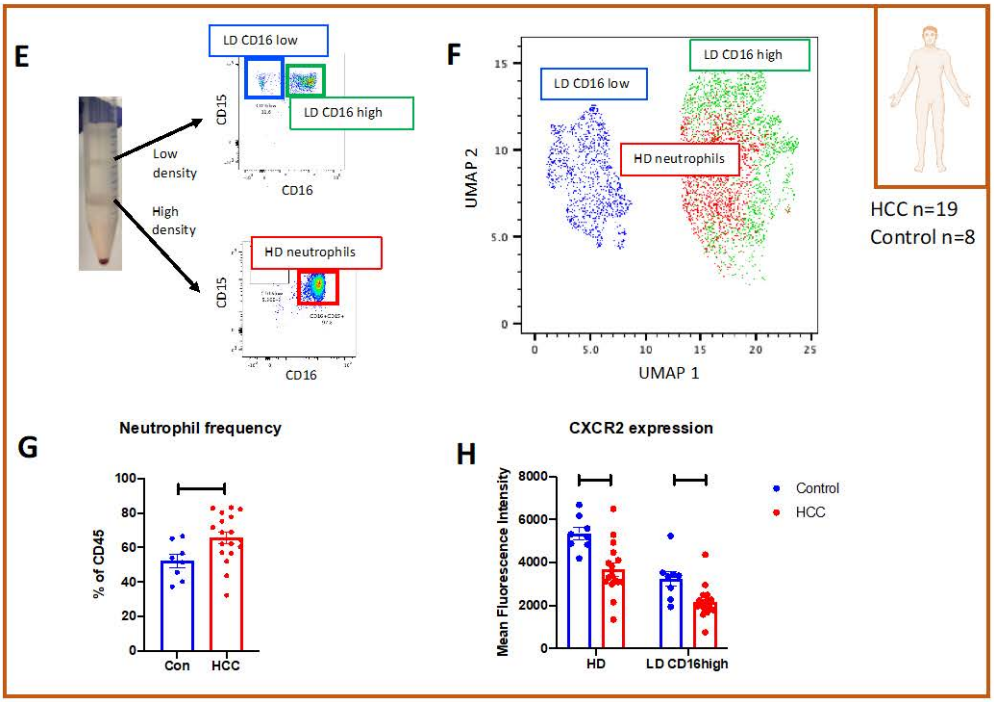
Method: *In vivo model*. High fat diet induced steatohepatitis plus orthotopic tumour implantations were conducted in C57BL/6J mice. Sham surgeries were conducted for controls. Neutrophils were isolated from the bone marrow, blood, spleen, liver and tumours. *Patient study*. HCC, chronic liver disease and healthy control patient blood samples were collected and neutrophils isolated by density gradient centrifugation. Neutrophils were analysed by flow cytometry.

Results: In the murine HCC model neutrophil frequency increased across all compartments with disease progression (**A**). HCC induced altered granulopoiesis with a preferential increase in early neutrophil progenitor populations such as granulocyte-monocyte progenitor cells (GMPs) and pre-neuts (**B**). Overall neutrophil phenotype mainly corresponded to the tissue of residence and maturation (**C**), however across all compartments HCC gave rise to a more immature neutrophil phenotype as demonstrated by a reduction in Ly6G expression (**D**). There were also HCC associated changes in neutrophil immune checkpoint and chemokine receptor expression. In patient blood samples 3 neutrophil populations were identified; low density (LD) CD16 low, LD CD16 high and high density (HD) neutrophils (**E**). These populations could be distinguished based on activation marker and chemokine receptor expression (**F**). Similar to the murine model there was an increase in neutrophil frequency in HCC patients compared to controls (**G**) with HCC neutrophils displaying a more immature phenotype as demonstrated by a reduction in CXCR2 expression (**H**).

Conclusion: HCC induces large shifts in neutrophil dynamics starting during granulopoiesis causing an increase in neutrophil frequency and giving rise to a more immature neutrophil phenotype. We hypothesize that these immature neutrophils have tumour promoting functions, utilized by the HCCs to promote tumour progression. Manipulation of neutrophil maturation may represent an important immune-therapeutic strategy.

Figure:


A
Neutrophil frequency
% of CD45
Sham
HCC
Blood (LD)
Blood (HD)
Spleen
Liver
B
Bone marrow neutrophil progenitor frequencies
****
**
GMPs
Pre-neuts
HCC n=5
Sham n=9
C
Blood - LD
Spleen
Blood - HD
Tumour
Liver
Maturation
Bone marrow
UMAP 2
UMAP 1
D
Ly6G expression
Mean Fluorescence Intensity
BM
Blood (LD)
Blood (HD)
Spleen
Liver


E
LD CD16 low
LD CD16 high
Low density
High density
CD15
CD16
HD neutrophils
F
LD CD16 low
LD CD16 high
HD neutrophils
UMAP 2
UMAP 1
HCC n=19
Control n=8
G
Neutrophil frequency
% of CD45
Con
HCC
H
CXCR2 expression
Mean Fluorescence Intensity
Control
HCC
HD
LD CD16high

PO-73
Tumour intrinsic and extrinsic role of Axl in models of hepatocellular carcinoma

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Background and Aims: The receptor tyrosine kinase Axl was identified as a major driver of tumour progression and metastasis in various cancer entities. In patients suffering from hepatocellular carcinoma (HCC), overexpression of Axl correlates with poor survival. The tumour intrinsic and extrinsic role of Axl in HCC is poorly understood. Therefore, we aim to better understand the impact of Axl in tumour and stroma cells.

Method: To study the role of tumour intrinsic Axl expression, we used Ras-transformed hepatocytes isolated from $p19^{ARF-/-}$ $Axl^{-/-}$ mice. Cells were analysed for invasive abilities *in vitro* and transplanted either subcutaneously or intravenously into mice to examine metastasis. Primary tumour burden and metastatic colonization were investigated. Liver tumours induced by diethylnitrosamine (DEN) and CCl_4-treated $Axl^{+/+}$ and $Axl^{-/-}$ mice were subjected for tumour-specific immune cell profiling by immunohistochemistry and gene expression profiling

Results: Axl expression promoted invasion of neoplastic hepatocytes but did not affect proliferation *in vitro* and *in vivo*. Axl reduced overall survival of mice and increased the number of pulmonary metastatic colonies.
In DEN/CCl_4- induced $Axl^{-/-}$ mice, liver tumour burden was enhanced by showing higher rates of hepatocytic proliferation. Interestingly, these tumours were less infiltrated with cytotoxic CD8+ T cells and granzyme B+ cells. Additionally, we observed an increased infiltration of FoxP3+ regulatory T cells and F4/80+ macrophages as well as elevated expression of M2-macrophage-specific *Mrc-1*, *Fizz-1* and *Tgf-beta1* suggesting that these tumours shifted towards an immunosuppressive environment. PD-L1 levels did not differ between $Axl^{+/+}$ and $Axl^{-/-}$ tumours, revealing alternative immune escape mechanisms. In accordance, analysis of HCC patient datasets showed a positive correlation of Axl expression with cytotoxic immune gene signatures

Conclusion: Our data suggest that tumour intrinsic Axl promotes cancer invasion and poor survival of mice. However, Axl expression in the microenvironment favours an anti-tumorigenic and pro-inflammatory response. Together, our findings show that Axl plays a crucial role in the dissemination of HCC and highlight the importance of Axl expression in the tumour microenvironment.

PO-76
Transarterial chemoembolization for hepatocellular carcinoma in clinical practice: temporal trends and survival outcomes over the last three decades in Italy

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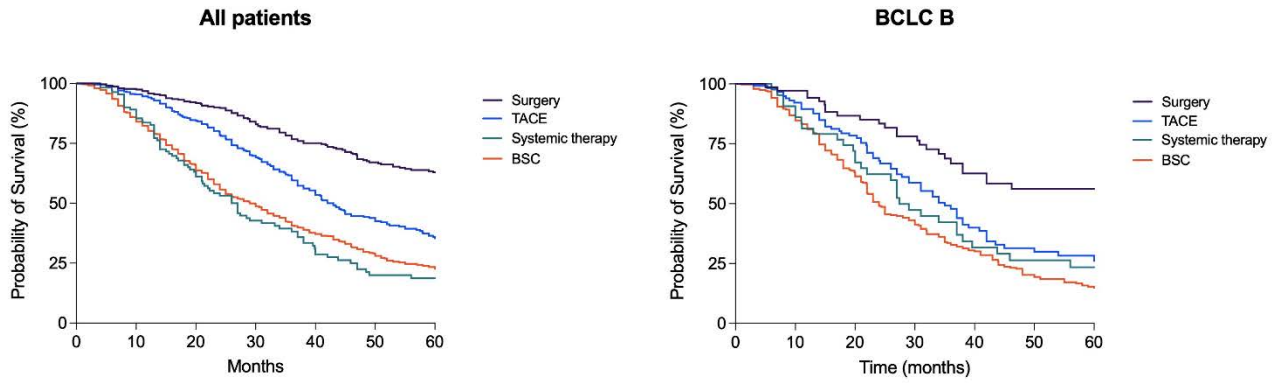
Background and Aims: Transarterial chemoembolization (TACE) is one of the most frequently applied treatments for hepatocellular carcinoma (HCC) worldwide. In this study, we aimed at evaluating whether and how TACE application and the survival of TACE-treated patients have changed over the last three decades in Italy.

Method: Data of 7,184 patients with HCC registered in the Italian Liver Cancer (ITA.LI.CA) database were analysed. Patients were divided in six groups according to the period of diagnosis: P1 (1988-1993), P2 (1994-1998), P3 (1999-2004), P4 (2005-2009), P5 (2010-2014), and P6 (2015-2019). All the analysis were repeated in the overall patient population and in Barcelona Clinic Liver Cancer (BCLC) B patients, who are the subgroup of HCC patients supposed to receive TACE according to guidelines. TACE was either defined as the first or the main (conferring the highest survival benefit) treatment.

Results: The proportion of patients receiving TACE as first or main therapy declined over time, and less than 50% of BCLC B patients were treated with chemoembolization from P3 onwards. Conversely, TACE was widely used even in patients belonging to other BCLC stages. Survival of TACE-treated patients (overall and in BCLC B) progressively increased from P1 to P6, and this improvement of prognosis was confirmed after adjustment of confounders. Even though the majority of patients in all time cohorts received only one TACE, there was an increasing proportion of those receiving 2 or ≥3 treatment sessions over time. The overall survival (OS) of patients undergoing repeated treatments was significantly higher compared to those managed with a single TACE (median OS 40.0 vs. 73.0 vs. 70.2 months in 1, 2 and ≥3 TACE groups, respectively; $p < 0.0001$). The majority of patients in these three groups died from tumour progression, and the proportion of deaths from liver decompensation in patients treated with 2 (19.3%) and ≥3 TACE (19.9%) was similar to that of patients receiving only 1 TACE (20.2%). After the first-line TACE, the adoption of curative therapies provided higher survival benefit than iterative TACE (83.0 vs. 42.0 months; $p < 0.0001$) that, in turn, provided longer survival compared to shifting to systemic therapies or best supportive care (Figure).

Conclusion: Despite a decline over time of the percentage of treated patients, TACE still has an important role in the therapeutic management of HCC patients. Survival in TACE-treated patients gradually improved over time, probably as a result of a better patients' selection. Iterative TACE is effective, but an up-ward shift to curative therapies provides better outcomes while transition to systemic therapies and best supportive care leads to a worse prognosis.

Figure:


All patients
Probability of Survival (%)
100
75
50
25
0
0
10
20
30
40
50
60
Months
Surgery
TACE
Systemic therapy
BSC
BCLC B
Probability of Survival (%)
100
75
50
25
0
0
10
20
30
40
50
60
Time (months)
Surgery
TACE
Systemic therapy
BSC

PO-94
PRAME is a novel target of Axl signaling in hepatocellular carcinoma progression

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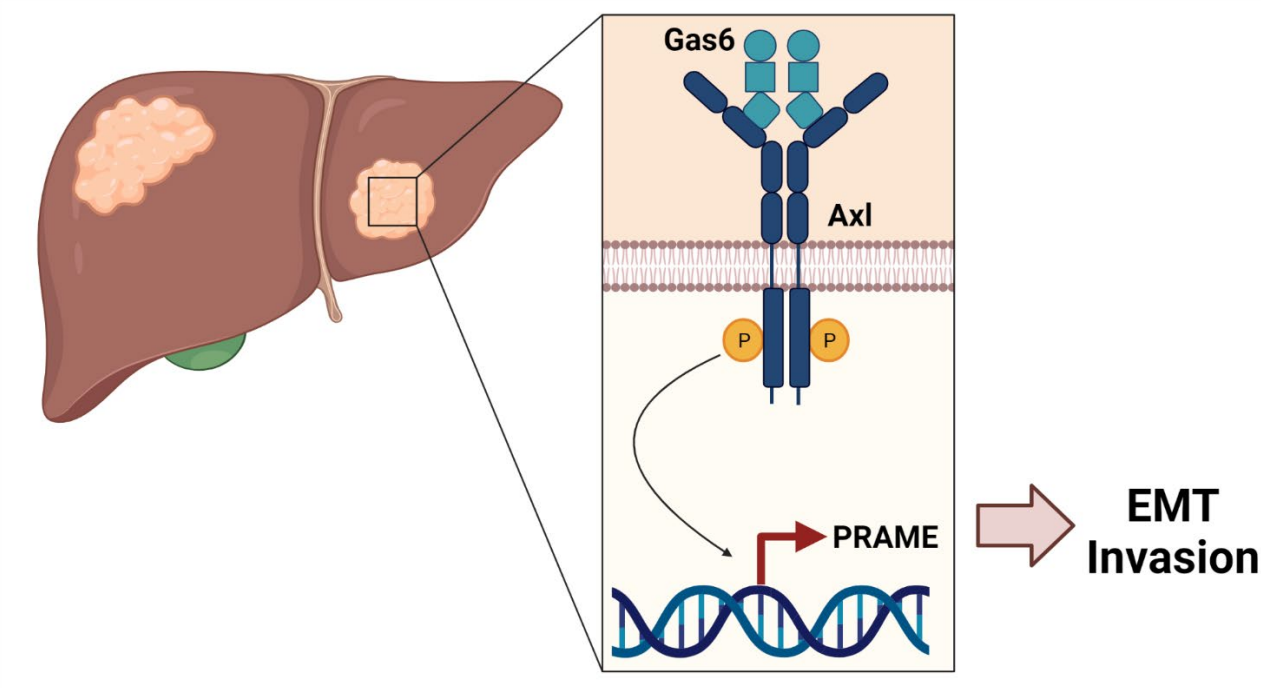
Background and Aims: The receptor tyrosine kinase Axl and its ligand Gas6 foster oncogenic effects during epithelial to mesenchymal transition (EMT) of hepatocellular carcinoma (HCC) which is accompanied by increased mortality of patients. The intrinsic function of Axl signaling in neoplastic hepatocytes and its pathophysiological consequences are poorly understood at current stage of research. In this study we aimed to identify targets of Axl signaling in HCC by exploiting well-characterized human HCC tumour models.

Method: Human EMT-transformed HCC cells expressing high Axl levels or harbouring CRISPR/Cas9-mediated Axl deficiency, the latter in absence or presence of Axl reconstitution, were subjected to Gas6 stimulation and subsequent RNA-seq analysis. Bioinformatics and VENN relations were applied to identify novel targets of the Gas6/Axl axis. Expression of Gas6/Axl targets was assessed in publicly available HCC patient datasets and in HCC tissue microarrays. *In vitro* analysis was performed including loss of functions studies to unravel target genes contribution to EMT and HCC progression

Results: RNA-seq analyses revealed PRAME and C15orf48 among others as novel targets of Gas6/Axl. Notably, these target genes showed elevated expression levels in Axl-stratified HCC patients and significant impact on patient survival. Pharmacological or genetic interference with Axl signaling resulted in strongly reduced expression of PRAME and C15orf48, suggesting *bona fide* targets of Gas6/Axl signaling. PRAME expression positively regulated 2D cell migration and invasion of 3D hepatospheres while proliferation and survival of HCC cells remained unaffected by altered PRAME levels. In accordance, PRAME expression induced by the Gas6/Axl/Mek/Erk1/2 signaling axis strongly associated with EMT in human HCC. Intriguingly, HCC patients expressing PRAME correlated with Axl expression and vascular invasion in a large proportion of HCC patients.

Conclusion: Our study demonstrates that PRAME is a novel target of Gas6/Axl signaling in HCC linked to EMT. Translation of *in vitro* findings into the HCC patient situation provides first insights that PRAME plays a crucial role in HCC progression.

Figure: Activation of the receptor kinase Axl by Gas6 in HCC cells induces PRAME expression, which positively regulates invasion and EMT.



PO-98
Effect of liver stiffness on hepatocellular carcinoma phenotype in a biomimetic 3D model

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Background and Aims: Hepatocellular carcinoma (HCC) usually occur in patients with an underlying chronic liver disease. The tumour micro-environment (TME) is therefore characterized by an increased deposition of extracellular matrix proteins (ECM). The abundance of ECM results in physical and biomechanical changes in the TME, which could influence tumour behaviour. The aim of our study was to determine how liver stiffness affects the HCC phenotype in a novel biomimetic 3D model to study tumour-stroma interactions.

Method: HepG2 and Huh7 cells were grown in a 3D biomimetic hydrogel model with tuneable biomechanical properties for 21 days. Matrix stiffness, epithelial-to-mesenchymal transitions (EMT), metastatic potential, drug sensitivity and overall survival was assessed and compared to current two-dimensional (2D) models, an *in vivo* HCC mouse model and clinical data. Tumour nodules were collected from the culture medium and cells were extracted from the gels for subsequent RT-qPCR analyses.

Results: Cells were embedded in hydrogels mimicking the onset of a fibrotic and cirrhotic TME, as determined by patient-derived data. HepG2 and Huh7 cells grown in a stiffer matrix (resembling a cirrhotic liver) increased proliferation and protein content, compared to those grown in a less stiff matrix (resembling a fibrotic liver). HepG2 cells significantly decreased albumin synthesis in the cirrhotic TME compared to the fibrotic TME, while Huh7 cells increased albumin synthesis. While urea production in HepG2 cells decreased over time, it increased in Huh7 cells in both conditions. Tumour nodules started to form spontaneously outside the 3D gels after 11 days. The HepG2 tumour nodules appeared earlier in the medium outside the cirrhotic gels and were significantly larger in size when compared to those found in the medium outside a fibrotic gel. Cells derived from these tumour nodules showed an increased mRNA expression of aSMA and SNAIL and decrease in E-cadherin when comparing cirrhotic to fibrotic environment, thus suggesting that increased stiffness could induce EMT in HCC cells. HepG2 cells grown in the 3D cirrhotic group were also more resistant to doxorubicin treatment compared with those grown in the 3D fibrotic group or grown in 2D. No differences were seen in drug distribution and penetrations between the two different gel compositions, thus further confirming that stiffness induced changes in the HCC phenotype that could explain a reduced response to chemotherapeutics. This was then confirmed by an increased expression of the drug resistance gene MDR1 in HepG2 cells grown in a cirrhotic gel, compared to those grown in a fibrotic gel.

Conclusion: Increasing the stiffness of the liver markedly increases the formation of metastatic nodules, induces several markers of EMT and reduces response to chemotherapeutics in a biomimetic 3D model of HCC.

PO-102

Inhibiting IRE1-a endonuclease activity potentiates the effect of doxorubicin in hepatocellular carcinoma.

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Background and Aims: Hepatocellular carcinoma (HCC) is the most common form of liver cancer, characterized by a high resistance to chemotherapeutic agents, such as doxorubicin (DOX). One potential contributing factor to this drug 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 tumours. However, its role in HCC remains unclear and requires further investigation.

Method: Publicly available data from the Human Protein Atlas (HPA) was used to find correlated expression of drug resistance/ER-stress markers in HCC-patients. A chemically induced mouse model for HCC was used and mice were treated twice per week with the ER-stress inhibitor 4u8C and/or DOX for 3 weeks, after the occurrence of tumours. Liver samples were taken for histological and molecular biology analyses.

Results: Data-mining of the HPA revealed that ER-stress markers are highly intertwined with different drug-resistance mechanisms in HCC-patients. 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 tumours, with the strongest reduction in the 4u8C+DOX combination treatment. Staining for Ki-67 showed a significant increase in the number of Ki-67 positive cells in mice with HCC, which was significantly reduced in all treatment groups, with the strongest reduction in the 4u8C+DOX combination treatment. In mice with HCC, 4u8C and the combination of 4u8C and DOX significantly increased apoptosis compared to untreated mice. Interestingly, no increase of apoptosis was seen in the DOX-treated group, which could suggest an alternative form of cell death. As DOX is known to induce ferroptosis, Transferrin Receptor-staining in liver tissue was quantified. This showed that DOX increased the area of Transferrin Receptor positive staining, suggesting a possible increase in ferroptosis. Interestingly, treatment with 4u8C reduced this effect, which is in line with previous findings that ferroptosis is – at least in part – dependent on ER-stress pathways.

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. The long-term impact of our study could open the possibility of ER-stress inhibitors as adjuvant treatments for HCC-patients.

PO-107
Ouctome of hydrodissection radiofrequency ablation in patients with pericholecystic hepatocellular carcinoma

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Background and Aims: Radiofrequency ablation (RFA) is a first-line curative treatment for very early/early-stage unresectable hepatocellular carcinoma (HCC). Pericholecystic HCC is one of the most difficult-to-treat lesions with RFA, due to the high risk of thermal-induced cholecystitis and biliary tract damage, with a reported risk of complication as high as 46%. We report a case series of 11 patients with pericholecystic lesions treated with RFA after tumour hydrodissection from the cholecystic wall with the injection of glucose solution (H-RFA) to minimize collateral thermal lesions.

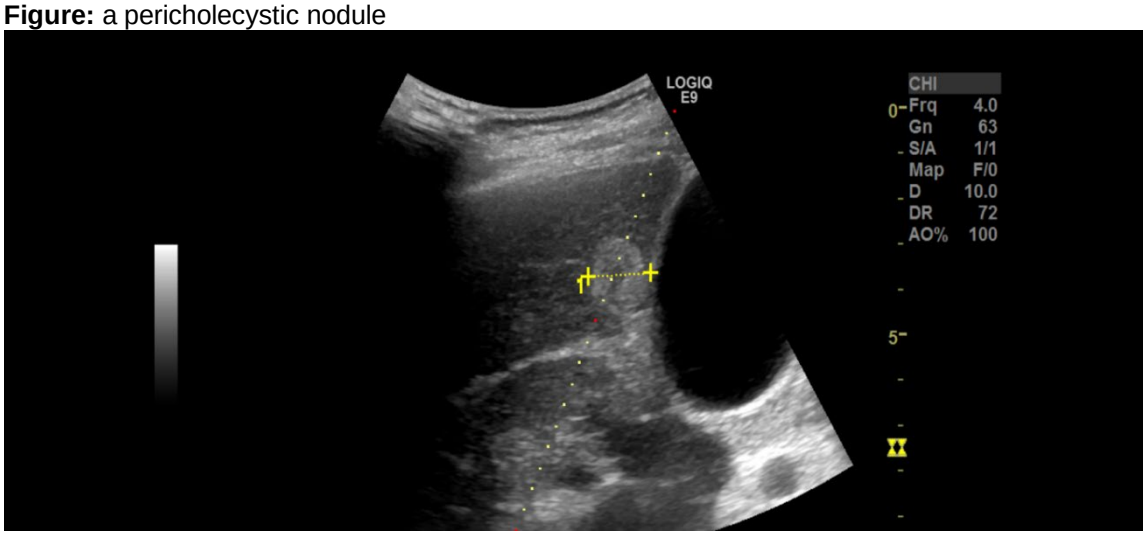
Method: We retrospectively reviewed 91 patients who underwent this RFA at our institution between January 2014 and December 2020 and followed up for a median (range) of 21 (2-63) months. The 11 patients treated for pericholecystic HCC were included in the study. They were judged not amenable to hepatic resection by the multidisciplinary board. Median age was 68 (35-86) years. The HCC was single with a median size of 20.5 (12-30) mm. Child-Pugh score was A in 8 (73%) and B in 3 (27%). A post-procedural contrast-enhanced ultrasonography (CEUS) was performed, and it was repeated one month later in all patients.

Results: No patient complained peri-procedural adverse events. The median overall survival was 21 (2-63) months, with 8 patients (72.7%) alive at the end of follow-up. Three patients (27.2%) died (one from liver failure, one from tumour progression and one from heart attack); three patients underwent liver transplantation.

The median recurrence-free survival was 3 (1-37) months. Nine (81.8%) patients showed a complete response (CR) at the one-month CEUS, while two patients (18.2%) showed a *persistence* of viable tumour; one was retreated with standard RFA achieving a CR, the other was not retreated over the follow-up. CR became “sustained” in only 2 patients (18.2%) retested at imaging performed 17 and 37 months later; in both cases nodules were <15 mm. Five patients (45.5%) showed an early local recurrence (within 4 months), one at 6 and another at 8 months patients.

Conclusion: H-RFA procedure is safe, but the oncological radicality is difficult to achieve and the tumour size greatly affects the result. However, local recurrences are frequently amenable to retreatment. H-RFA enriches the therapeutic armamentarium for unresectable pericholecystic HCCs and can act as bridge and downstaging therapy in patients awaiting liver transplant.

Figure: a pericholecystic nodule



PO-119

Efficacy of combination therapy with lenvatinib, programmed cell death 1 inhibitors and transarterial therapy: a propensity score-matching cohort study

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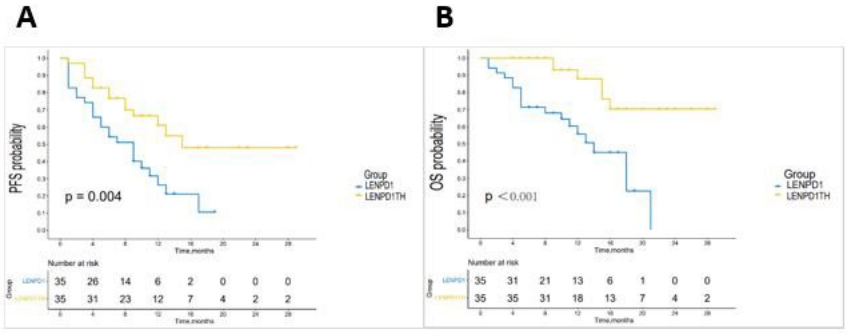
Background and Aims: Despite showing efficacy as separate treatments, few studies have investigated the efficacy of triple combination therapy with lenvatinib, programmed cell death 1 (PD-1) inhibitors and transarterial therapy in patients with intermediate/advanced hepatocellular carcinoma (HCC) compared to combination therapy with lenvatinib and PD-1 inhibitors.

Method: We retrospectively analysed data from patients with intermediate to advanced HCC treated at Tianjin Cancer Hospital from December 2018 to October 2020 and compared patients who received lenvatinib combined with PD-1 inhibitors and transarterial therapy(LEN-PD1-TH) with those who received lenvatinib and PD-1 inhibitors(LEN-PD1). Propensity score-matching was used to account for differences in baseline characteristics. The primary study endpoint was progression-free survival estimated using the Kaplan-Meier method. Secondary endpoints were overall survival and overall response rate assessed using modified Response Evaluation Criteria in Solid Tumours. The Cox proportional hazards model was used to identify factors that affected prognosis.

Results: A total of 152 patients were included. Propensity score matching led to 35 matched patients in each group. The median follow-up time was 14 months (95% CI, 10.1-17.9) as of May 2021. The lenvatinib/PD-1/transarterial therapy group had a longer median progression free survival and overall survival versus the lenvatinib/PD-1 group (15 vs. 9 months; $p = 0.004$ and NA vs. 14 months; $p < 0.001$, respectively). The overall response rate was significantly higher in the lenvatinib/PD-1/transarterial therapy group versus the lenvatinib/PD-1 group (42.9 vs. 20.1%; $p = 0.039$). Multivariate analysis showed that all triple therapy regimens were associated with improved progression-free (hazard ratio [HR]=0.362; 95% CI, 0.185-0.771; $p = 0.003$) and overall (HR=0.143; 95% CI, 0.053-0.383; $p < 0.001$) survival. Child-Pugh class B and Barcelona clinic liver cancer stage C disease were identified as risk factors for progression free survival, and large tumour size was a risk factor for overall survival. Patients with extrahepatic metastases, no vascular invasion, tumour diameter < 10 cm, tumour number > 3, BCLC stage C, and alpha-fetoprotein ≤ 400 ng/ml showed a tendency to benefit from the triple therapy regimen. The addition of transarterial therapy to lenvatinib and PD-1 inhibitors did not significantly increase adverse reactions.

Conclusion: Lenvatinib combined with PD-1 inhibitors and transarterial therapy improved survival outcomes for patients with intermediate to advanced HCC compared to lenvatinib and PD-1 inhibitors alone.

Figure:


A
B
PFS probability
p = 0.004
Group
LENPD1
LENPD1TH
Number at risk
35 26 14 6 2 0 0 0
35 31 23 12 7 4 2 2
OS probability
p <0.001
Group
LENPD1
LENPD1TH
Number at risk
35 31 21 13 6 1 0 0
35 35 31 18 13 7 4 2

PO-120
Hepatocellular carcinoma tumour burden in the GAN diet-induced obese mouse model of NASH with advanced fibrosis

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Background and Aims: Non-alcoholic steatohepatitis (NASH) predisposes to the development of severe fibrosis and hepatocellular carcinoma (HCC). Preclinical animal models resembling NASH-driven HCC development are important tools for exploring novel pharmacological interventions for HCC. The present longitudinal study aimed to characterize disease progression in the GAN (Gubra-Amylin NASH) diet-induced obese (DIO) mouse model of NASH.

Method: Male C57Bl/6J mice were fed the GAN diet (40 kcal-% fat, 22% fructose, 10% sucrose, 2% cholesterol) for up to 88 weeks (n=12-15/group). Liver disease progression in DIO-NASH mice was evaluated using the clinical NAFLD Activity Score (NAS) and fibrosis staging system, quantitative histology, macroscopic tumour quantification and transcriptomics. Liver tumour histopathological classification was performed by an expert clinical pathologist.

Results: DIO-NASH animals demonstrated progressive NASH (NAS≥5) and fibrosis (stage≥F1) from 28 weeks of GAN diet feeding. Notably, advanced fibrosis (stage F3, bridging fibrosis) and macroscopic liver tumour development was observed in ≥85% of animals from 58 weeks of GAN diet feeding. Liver tumours demonstrated consistent architectural and cytologic features of HCC, notably loss of reticulin-stained fibres. Progressive HCC development was supported by increased quantitative histological markers of proliferation (Ki67), hepatic biliary/progenitor cells (CK7, CK19) and angiogenesis (CD31). Disease progression was further highlighted by increased quantitative histological markers of steatosis (lipids, hepatocytes with lipid droplets), inflammation (number of inflammatory foci, galectin-3), fibrosis (PSR, collagen 1a1), activated stellate cells (α-SMA). Finally, hepatic whole-tissue transcriptome signatures demonstrated enhanced fibrogenesis (collagens, matrix metalloproteins) and tumorigenesis activity (cell cycle control, growth factors).

Conclusion: DIO-NASH mice show advanced NASH with severe fibrosis and high HCC incidence. The extended GAN DIO-NASH-HCC mouse model is suitable for profiling novel drug therapies for advanced fibrosing NASH and HCC.

PO-122
Unrecognised liver cirrhosis: common and associated with worse survival in patients diagnosed with hepatocellular carcinoma – a Swedish nationwide cohort study

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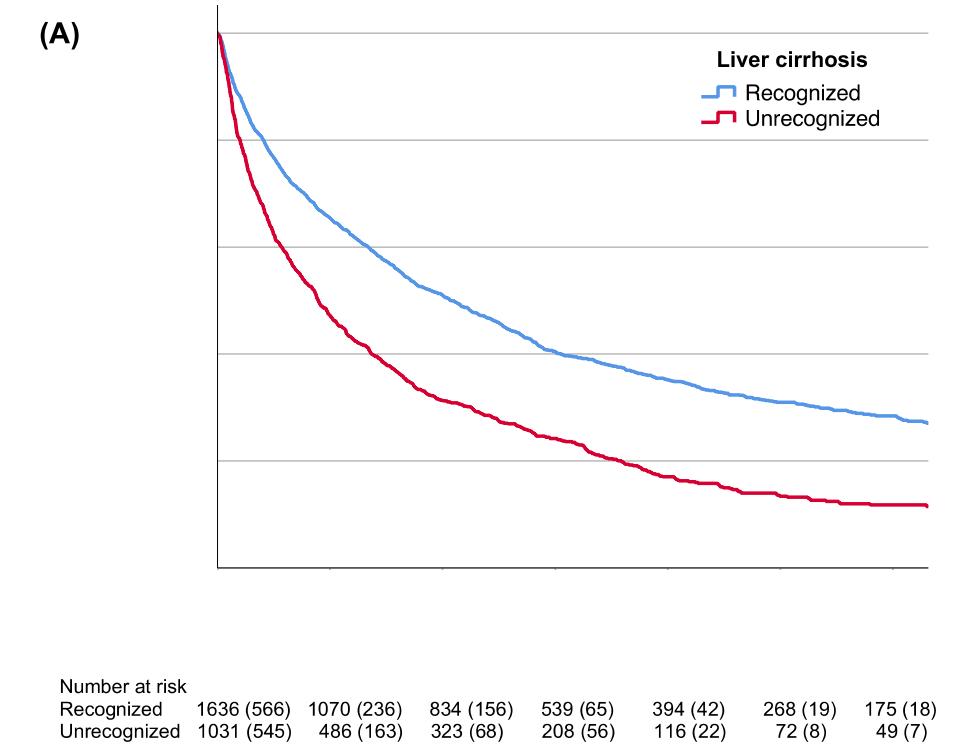
Background and Aims: Studies examining the importance of unrecognised liver cirrhosis (LC) in patients diagnosed with hepatocellular carcinoma (HCC) are scarce. We aimed to determine the extent to which LC is unrecognised in patients diagnosed with HCC, and to estimate the associations between unrecognised LC and stage of HCC at diagnosis, expected survival, and mortality risk.

Method: Using the Swedish quality registry for liver cancer (SweLiv), we identified all adults who received a diagnosis of HCC in Sweden between 2012 and 2018. Baseline data, including HCC stage (Barcelona Clinic Liver Cancer [BCLC]), were retrieved. Using SweLiv and other nationwide Swedish registries, we identified patients with LC among HCC patients. The follow-up time was ended at the date of death, emigration from Sweden, or December 31st 2020; whichever occurred first. Using Cox proportional-hazards regression modelling, we calculated unadjusted and adjusted (sociodemographic variables, year of HCC diagnosis, LC etiology, BCLC stage) hazard ratios (HR) for death for patients with unrecognised LC compared to those with recognised LC (reference group).

Results: Of 3473 patients, 2670 (77%) were regarded as having LC. Of these, 1033 (39%) lacked ongoing LC care, hence being regarded as unrecognised LC. Patients with unrecognised LC were more often male (83% vs 75%), older (median age 69 vs 66 years), and had lower educational level (low level 41% vs 36%). Surveillance detected 55% of HCC cases in patients with recognised LC. Unrecognised LC patients had larger tumours (median 5.5 cm vs 3.0 cm), more multinodular cancer (22% vs 12%), and heavier regional (18% vs 7.8%) and distant (23% vs 9.5%) metastasis burden. Consequently, unrecognised LC patients were seldom diagnosed with HCC at early-stage (BCLC 0-A, 19% vs 38%). Unrecognised LC was also associated with worse median survival (0.89 years, 95% confidence interval [CI] 0.78-1.01) compared to recognised LC (2.07 years, 95% CI 1.85-2.29) (Figure 1A). The unadjusted and adjusted HRs associated with unrecognised LC were 1.68 (95% CI; 1.54-1.84) and 1.54 (95% CI; 1.40-1.70), respectively. In subgroup analyses, a total of 858 patients (32%) received treatment with curative intention. Recognised LC patients accounted for most of these cases (75%). The median survival time after surgery was 6.17 years (95% CI; 5.89-6.45) for patients with recognised LC, and 4.81 years (95% CI; 4.39-5.23) for those with unrecognised LC (Figure 1B). The unadjusted and adjusted HRs for unrecognised LC were 1.50 (95% CI; 1.19-1.91) and 1.45 (95% CI; 1.10-1.91), respectively.

Conclusion: LC is frequently unrecognised in patients diagnosed with HCC. Patients with unrecognised LC are diagnosed at more advanced stage HCC, and have worse overall survival. These findings support prior observations, and underline the importance of early LC recognition for timely HCC detection.

Figure:



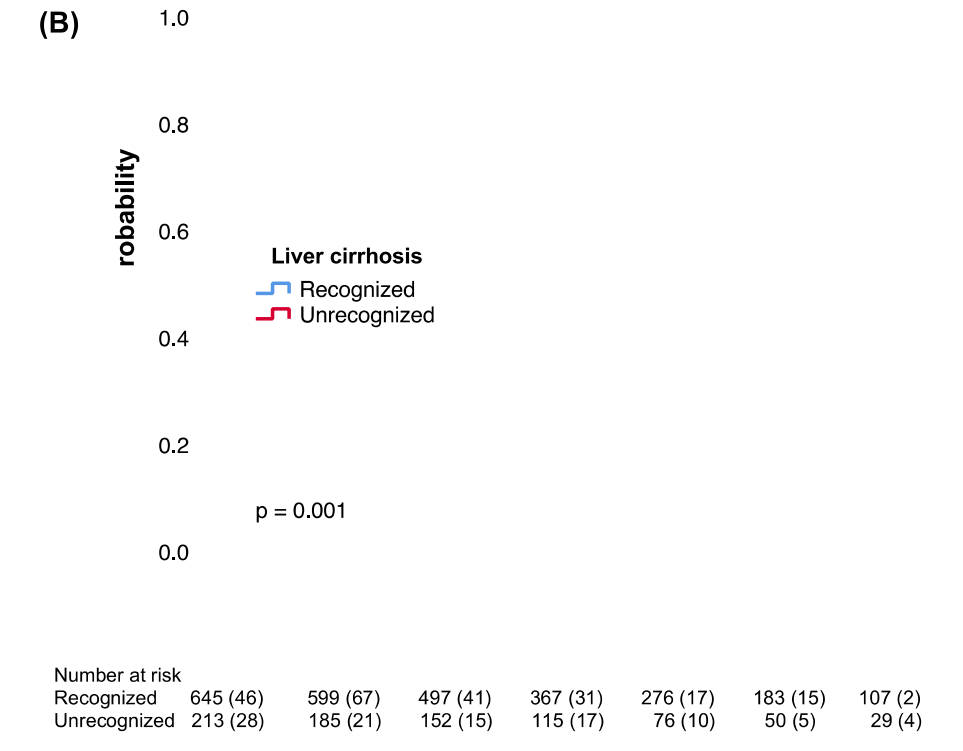


Fig 1. Kaplan-Meier survival curves in a cohort of 2670 patients diagnosed with hepatocellular carcinoma (HCC) in Sweden (2012-2018). Survival probabilities compared by liver cirrhosis status (recognized vs unrecognized) (A). A subgroup analysis of 858 patients who received surgical treatment with curative intention is also shown (B). The follow-up time was ended at the date of death, emigration from Sweden, or December 31st 2020; whichever occurred first. Number of terminal events are shown in parentheses. Time after HCC (A) and surgery (B) was limited to 6 years.

PO-126
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 tumours 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) were designed and synthesized. The antitumour effect of these compounds was evaluated by measuring their impact on the viability of human CCA cells (EGI-1 and HUCCT1), newly generated CisPt-resistant EGI-1 CCA cells and normal human cholangiocytes (NHC). The DNA damage induced by the two best candidates (Aurki-Pt#1 and #2) was assessed using comet assay. To ascertain the uptake of Aurki-Pt#1 and #2 by cancer cells, indirect competition studies of known fluorescent substrate using flow cytometry and direct accumulation studies using HPLC-MS/MS were carried out. Antitumour effect of Aurki-Pt#1 and #2 was tested *in vivo* using a subcutaneous xenograft model of CCA.

Results: Aurki-Pt#1 and #2 significantly reduced CCA cell viability. Both compounds induced higher DNA damage in CCA cells than CisPt, thus being more effective triggering apoptosis *in vitro*. Importantly, Aurki-Pt#1 and #2 also promoted cell death in CisPt-resistant CCA cells, while this lethal effect was absent in NHC in culture. On the other hand, Aurki-Pt#1 and #2 decreased the proliferation of those CCA cells that survived, but did not have any effect on NHC. Aurki-Pt#1 and #2 were taken up by the cells across OCT3 and OATP1A2. Finally, Aurki-Pt markedly hampered tumour growth of subcutaneously implanted CCA cells in mice 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, representing a promising therapeutic tool for naïve or CisPt-resistant CCA tumours.

PO-127
Fatty acid oxidation fuels the progression of highly proliferative cholangiocarcinoma cells

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Background and Aims: Cell metabolic reprogramming is a major hallmark of cancer, representing a potential target for therapy. However, the metabolic rewiring in cholangiocarcinoma (CCA) remains still unclear. Here, we aimed to investigate the preferential energy substrates of CCA cells, the metabolic-related processes, and their role in cancer cell proliferation.

Method: *The in vitro and in vivo* tumourigenic capacity of 5 human CCA cell lines was analysed. The uptake and complete oxidation into CO_2 of [^{14}C]-glucose, [^{14}C]-glutamine, and [^{14}C]-palmitate were investigated. Proteome, lipoprotein uptake, lipid content and their intracellular metabolic fate were evaluated in CCA cells and compared to 4 primary cultures of normal human cholangiocytes (NHC). The *Akt1*/*Nicd1*-driven CCA mouse model was also evaluated.

Results: The EGI1 CCA cell line showed the highest tumourigenic, proliferation and migration capacities. Metabolic studies in high (EGI1) *vs* low (HUCCT1) proliferative CCA cells *in vitro*, and in liver CCA tumour tissue from the *Akt1*/*Nicd1*-driven CCA mouse model were performed. Both EGI1 and HUCCT1 incorporated more oleate and palmitate than NHC, leading to increased triglyceride storage, also observed in *Akt1*/*Nicd1*-driven CCAs. The highly-proliferative EGI1 CCA cells showed greater uptake of fatty acids (FAs), very-low-density lipoproteins (VLDLs) and high-density lipoproteins (HDLs) and levels of transporters than control NHC and HUCCT1 CCA cells, which led to increased triglyceride synthesis and storage. Moreover, EGI1 CCA cells were characterized by increased FA oxidation (FAO), while glucose and glutamine uptake and oxidation remained unchanged vs NHC3 cells. The fatty acid oxidation (FAO) rate and related proteome enrichment was specifically upregulated in EGI1, and consequently, pharmacological blockade of FAO induced a more pronounced inhibition of their proliferative capacity compared to HUCCT1. In contrast, glucose and glutamine uptake and oxidation remained unchanged in EGI1 vs NHC3 while glutamine oxidation increased in HUCCT1. Notably, high immunoreactivity of the first enzyme involved in FAO, i.e., acyl-CoA dehydrogenase *ACADM*, was observed in human intrahepatic CCAs (iCCAs; 92.3% of tumours), while its expression was faint in normal bile ducts. The protein levels of ACADM in iCCAs correlated with the levels of the proliferation marker PCNA.

Conclusion: CCA cells are heterogeneous in terms of preferential energy source. Highly-proliferative human CCA cells rely on lipid and lipoprotein uptake to fuel FA catabolism, suggesting that inhibition of FAO and/or lipid uptake could represent a novel therapeutic strategy for patients in this CCA subclass.

PO-128

Progression patterns and therapeutic sequencing following immune checkpoint inhibition for HCC: an observational study.

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Background: Different approaches are available after progression of disease (PD) while on treatment with immune checkpoint inhibitors (ICI) for hepatocellular carcinoma (HCC), including continuation of ICI, switching to tyrosine kinase inhibitors (TKIs) and cessation of therapy. Little is known about progression patterns and their relationship with optimal sequencing and survival outcomes post-ICI.

Methods: From an international consortium of 13 tertiary-care referral centres, we screened 604 HCC patients treated with ICIs, including only those who experienced PD by data cut-off. We evaluated post-progression survival (PPS) according to treatment strategy at PD and verified its relationship with radiologic patterns of progression: intrahepatic growth (IHG), new intrahepatic lesion (NIH), extrahepatic growth (EHG), new extrahepatic lesion (NEH) and new vascular invasion (nVI).

Results: 364 (60.3%) patients had PD during observation, mostly following PD-1/PD-L1 monotherapy (80%). Median PPS was 5.3 months (95%CI: 4.4-6.9; 271 events). At data cut-off, 165 patients (45%) received no post-progression anticancer therapy. Both IHG (HR 1.64 [95%CI:1.21-2.22]; p=0.0013) and nVI (HR 2.15 [95%CI:1.38-3.35]; p=0.0007) at PD were significantly associated with shorter PPS. Continuation of ICI therapy beyond PD occurred in 64 patients (17.6%). Multivariate models adjusted for progression patterns, treatment line, and ALBI grade and ECOG-PS at PD confirmed receipt of ICI beyond PD with (HR 0.17, 95%CI 0.09-0.32; p<0.0001), or without subsequent TKI (HR 0.39, 95%CI 0.26-0.58; p<0.0001) as predictors of prolonged PPS compared to no anticancer therapy.

Conclusions: ICI-TKI sequencing is a consolidated option in advanced HCC, with poorer prognosis predicted by nVI and IHG. Despite lack of recommendation, continuation of ICI beyond progression in

HCC is adopted in clinical practice: efforts should be made to identify patients who benefit from this approach.

PO-129
Clinicopathological and survival features of hepatic lymphomas: a retrospective single-centre study

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Background and Aims: The incidence of hepatic lymphoma is increasing recently. Clinical presentation and radiological findings are nonspecific. Since patients require chemotherapy instead of surgical excision, an accurate histopathological diagnosis is essential. The aims of this study were to describe the main clinical and pathological characteristics of liver lymphomas and identify prognostic factors associated with poor outcomes.

Method: A retrospective study involving all patients with histological diagnosis of liver lymphoma between January 2010 and December 2019 was performed. Their main clinicopathological characteristics and prognostic factors were evaluated.

Results: A total of 36 patients were identified, with mean age of 56.6 years (±13.08); 21 (58%) were male. There were three patients with primary liver lymphoma (8.3%) and 33 with secondary lymphoma (91.7%), with involvement of other organs, most commonly lymph nodes (69.5%), bone marrow (52.8%) and spleen (41.6%). The most common histological subtype was large B-cell lymphoma (33.3%), followed by Hodgkin lymphoma (19.4%), Burkitt lymphoma (11.1%) and T-cell lymphoma (11.1%). The most common clinical manifestations included fever, lymphadenopathy, weight loss, fatigue, night sweats and abdominal discomfort; three patients (8.3%) were asymptomatic. Computed tomography scan revealed heterogeneous radiological patterns including a solitary nodule (26.5%), multiple nodules (41.2%) or diffuse infiltration (32.4%). Chemotherapy was administered to 29 patients, with complete response in 51.5%, partial response in 13.8% and absence of response in 34.5%; in 7 patients treatment was not started because of rapid disease progression. The mortality rate during follow-up was 55.6%. Higher levels of C-reactive protein (CRP) ($p = 0.031$) and absence of response to treatment ($p < 0.001$) were significantly associated with higher mortality.

Conclusion: We report one of the largest series of hepatic lymphomas to date. This is a rare clinical entity consisting of a heterogeneous group of lymphoproliferative disorders that may involve the liver as part of a systemic disease or, less commonly, be confined to this organ. Clinical presentations and radiological findings are often heterogeneous and non-specific. It is associated with high mortality and poor prognostic factors include higher levels of CRP and absence of response to treatment.

Figure: Computed tomography scan presentation patterns, including uninodular, multinodular or diffuse.

PO-132
Enhanced recovery after surgery: application of 2016 liver surgery guidelines in a middle volume western centre

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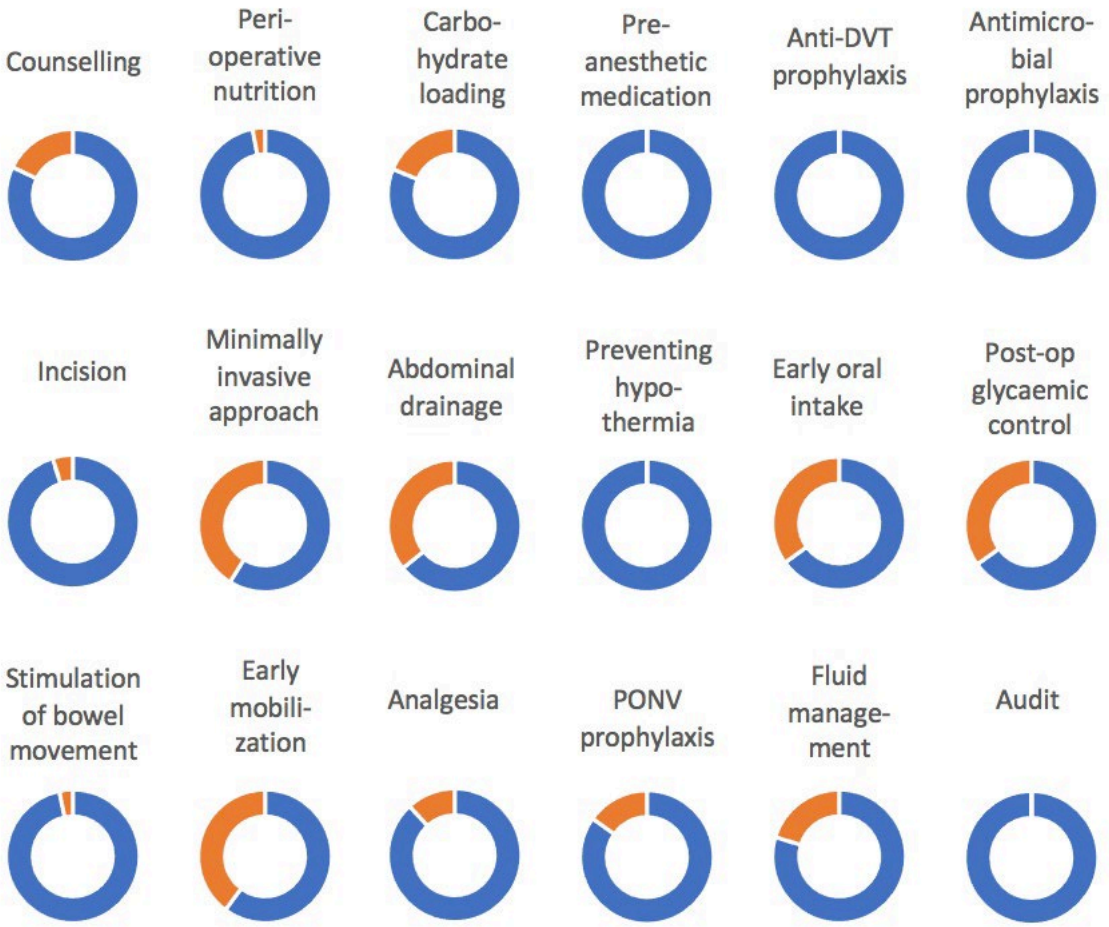
Background and Aims: Enhanced Recovery After Surgery (ERAS) is proved to reduce complications and postoperative length of hospital stay and to restore faster patients' preoperative conditions. However, adherence to ERAS in liver surgery is difficult to achieve due to deep-rooted traditional practices. In our middle volume centre for hepatic surgery we introduced ERAS in 2019, pursuing 18 of the 23 items identified by the 2016 guidelines.

Method: Data from all the consecutive patients who underwent elective liver surgery from January 2019 to July 2021 were retrospectively collected. Every patient followed the ERAS program. The primary outcome was successful completion of the ERAS program, defined as accomplishment of at least 80% of the items by the patient and the medical team. The secondary outcome was specific adherence to each ERAS item.

Results: 60.5% of the 129 included patients successfully completed the ERAS program. The items less likely to be completed were avoidance of abdominal drain in minor resections (64%), early oral intake (65%), post-operative glycaemic control (65%), and early mobilization (60%). After univariate and multivariate analysis length of surgery and ECOG performance status 1 were the only items significantly associated with ERAS program adherence (p=0.044). Mean duration of surgery was 440 minutes for the patients who dropped out from ERAS program, while it was 306 minutes for those who completed the program. Surgery complexity and extension did not associate significantly with ERAS failure.

Conclusion: ERAS application in liver surgery with a satisfactory adherence to the protocol can be achieved also in a Western middle volume centre. Improvements in patient post-operative assistance should be adopted, particularly in patients who undergo prolonged operative duration.

Figure:


Counselling
Peri-operative nutrition
Carbo-hydrate loading
Pre-anesthetic medication
Anti-DVT prophylaxis
Antimicro-bial prophylaxis
Incision
Minimally invasive approach
Abdominal drainage
Preventing hypo-thermia
Early oral intake
Post-op glycaemic control
Stimulation of bowel movement
Early mobili-zation
Analgesia
PONV prophylaxis
Fluid manage-ment
Audit

PO-135
Prominent pseudoacini in focal nodular hyperplasia: a potential diagnostic pitfall

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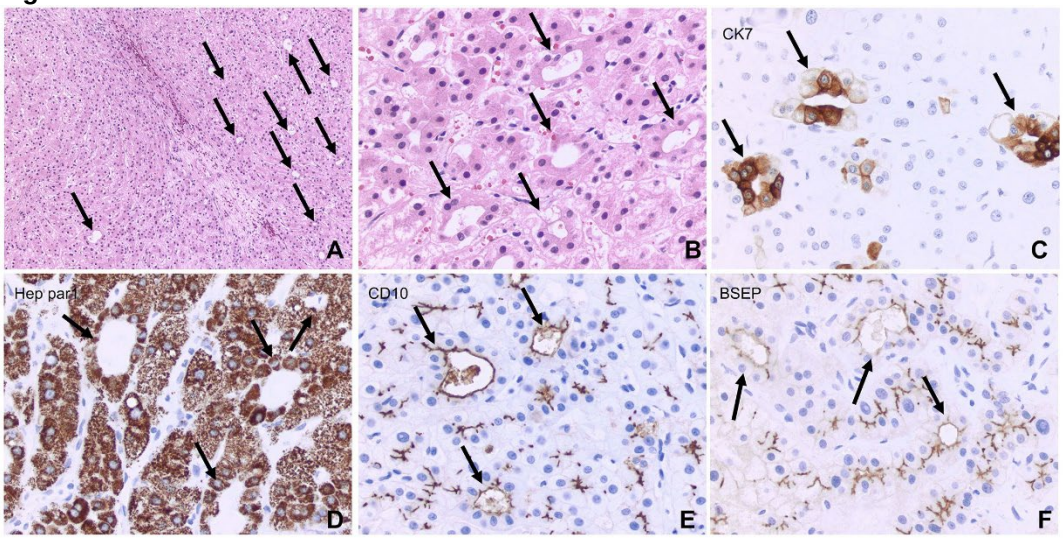
Background and Aims: The presence of pseudoacini (PA) in liver neoplasms is generally a feature of hepatocellular carcinoma (HCC) and occurs rarely in hepatic adenomas. However, rarely these can occur in focal nodular hyperplasia (FNH) and may pose diagnostic challenges, especially when numerous. The study was aimed to evaluate the occurrence of PA in FNH and study their clinicopathologic correlations.

Method: A total of 95 FNH cases diagnosed from 2005 to 2020 at our institution were included in the study. Demographic, clinical and detailed histologic features were analysed in each case, with special emphasis on the presence of PA. PA were recognised as a circular arrangement of hepatocytes with a centrally dilated lumen present within the lobular parenchyma. These were different from pseudorossettes, which are typically seen in the periseptal/periportal region, have a barely recognisable central lumen and lack accumulated secretion/bile. Cases with >3 PA/20X field in the most prominent area were recorded with prominent PA. Various mmunostains were evaluated with specific attention to Glutamine synthetase (GS), CK7, HepPar1, CD10 and BSEP.

Results: Among 95 FNH cases, 12 showed prominent PA (12.6%). Of these 2 were seen in men and 10 in women, and 3 occurred in patients >50y. The PA were numerous in 3 cases, leading to an initial consideration of HCC in the differential diagnosis. All cases showed typical geographic staining for GS and lacked any other features for HCC. Histologically, cells lining the PA were similar or slightly smaller compared to adjacent hepatocytes, and showed CK7 and Heppar-1 reactivity in all cases. The inner lumen of PA was highlighted by CD10 and BSEP immunostains (Figure). Bile or eosinophilic secretions could be seen in some of them. Of these 1 had prior biopsy, which was diagnosed as well differentiated hepatocellular neoplasm. No significant difference in clinical and pathologic features between cases with and without PA was noted.

Conclusion: PA can be seen in a subset of FNH cases and raises concern for HCC when seen in patients >50y, especially men. However, other clinicopathologic features including GS staining pattern are not different from typical FNHs. The findings suggest this is likely a manifestation of chronic cholestasis in the lesion, but the exact mechanism is unclear. This feature is a potential diagnostic pitfall on needle biopsies and awareness is needed to avoid misdiagnosing these as HCCs.

Figure:



PO-141
Investigating CXCR2 inhibition + anti-PD1 immunotherapy in a NASH-HCC mouse model using Imaging Mass Cytometry

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Background and Aims: Primary liver cancer is a leading cause of cancer related deaths and its most common form is hepatocellular carcinoma (HCC), which is rising in incidence and remains a disease of poor prognosis. Immunotherapy is becoming established as a front-line treatment for advanced HCC, however its efficacy is limited to a minority of patients. In particular, there is evidence that a background of non-alcoholic steatohepatitis (NASH) can reduce effectiveness of immune checkpoint inhibition. In this study we employ Imaging Mass Cytometry (IMC) to determine how co-therapy of anti-PD1 (Biolegend) and a small molecule inhibitor of the neutrophil chemokine receptor CXCR2 (AZD5069 – AstraZeneca) remodels the tumour microenvironment (TME) in a murine model of NASH-HCC.

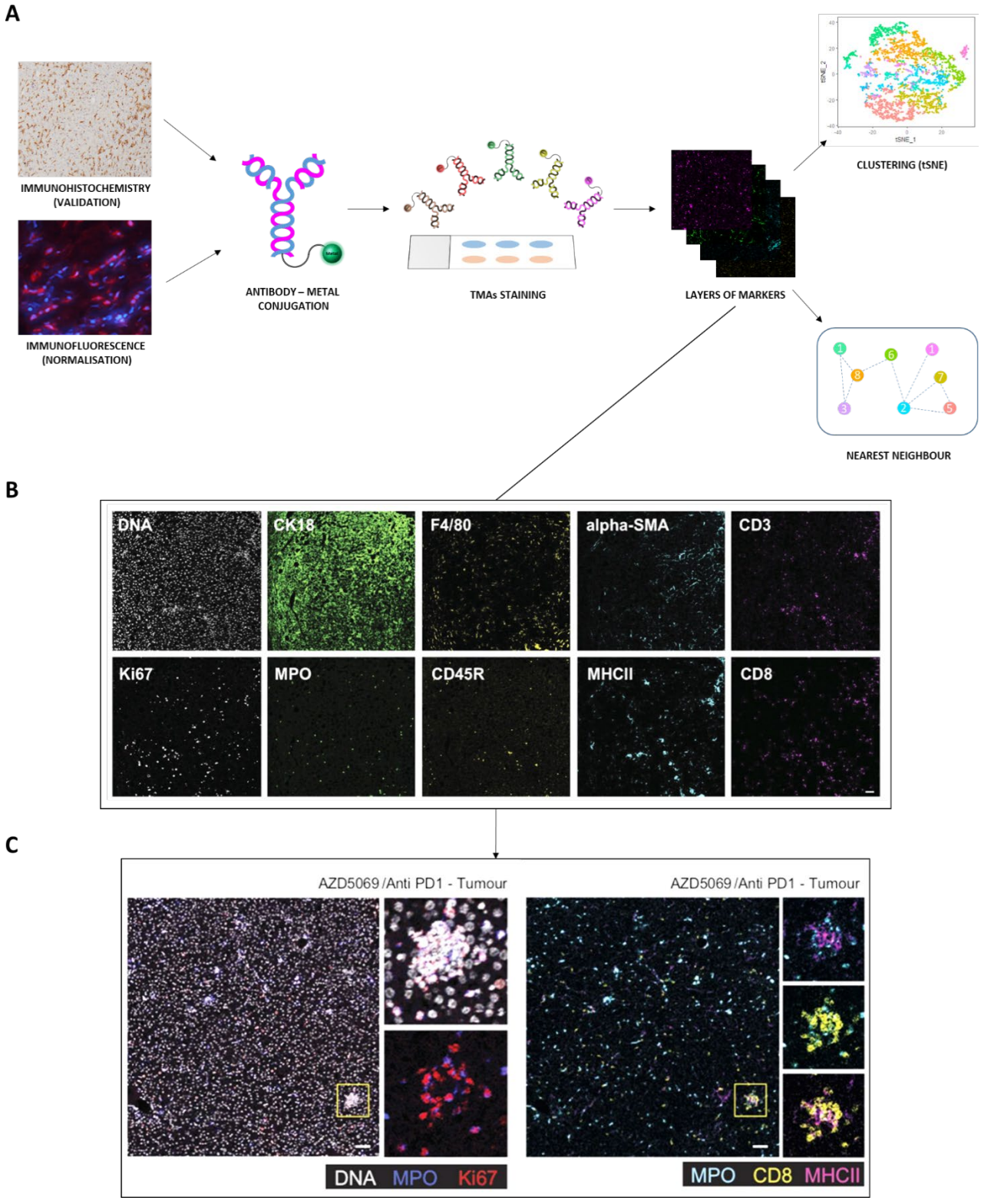
Method: Tumours were induced in C57BL/6 mice by intraperitoneal (IP) injection of Diethlynitrosamine (DEN) at 2 weeks of age. Mice were then fed the ALIOS diet (American Lifestyle-Induced Obesity Syndrome) from 8 weeks to 40 weeks to induce NASH. IgG control, anti-PD and AZD5069 were administered IP for the final 10 weeks. Tissue microarrays (TMAs) of liver tumours were analysed by IMC using the Hyperion system (Fluidigm). Antibodies directed to the TME were validated first by immunohistochemistry and immunofluorescence prior to being conjugated to metal isotopes. Single cell segmentation was performed using the Bodenmiller pipeline and analysis performed in HistoCat. Cell type abundance was characterised for all treatment groups and cell type interactions analysed by Nearest Neighbour Analysis. **(A)**

Results: IMC identified multiple cellular components of the NASH-HCC TME including lymphoid cells [T (CD3+, CD8+) and B cells], myeloid cells [neutrophils (MPO+), macrophages (F4/80+) and antigen presenting cells (MHC-II+F4/80-)], stromal cells (αSMA+), proliferating (Ki67+) cells and parenchymal/tumour cells (Cytokeratins) **(B)**. Most significantly, co-treatment with anti-PD1 and AZD5069 remodelled the TME to promote the formation of immune clusters containing Ki67+MPO+ proliferating neutrophils in direct contact with cytotoxic T cells and antigen presenting cells **(C)**. Immunohistochemistry identified the immune hubs to be associated with elevated expression of cytotoxic Granzyme B. These data correlated with therapeutic benefits including reduced tumour burden and improved survival.

Conclusion: IMC allows for the identification of multiple cell types in a spatial context and quantification of changes in cell-cell interactions within the TME. In this study, IMC demonstrated that combined anti-PD1 and CXCR2 inhibitor therapy in NASH-HCC encouraged the formation of cytotoxic immune hubs

containing locally proliferating neutrophils, CD8+ T cells and antigen presenting cells. We then propose that the manipulation of neutrophils may have therapeutic potential in NASH-HCC.

Figure:



PO-143
Hepatocellular carcinoma recurrence post Liver transplant

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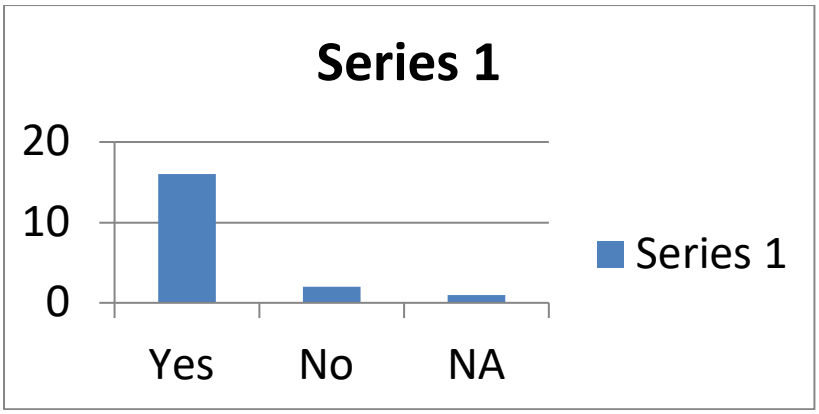
Background and Aims: Liver transplant offers the curative option for Hepatocellular cancer. There is strict criteria (Milan's criteria) followed for declaring eligibility for liver transplant in HCC. However 10 to 20% patients will still experience recurrence. Currently there are no guidelines in UK for surveillance in patients transplanted for HCC post liver transplant. However several international studies have identified factors significantly associated with recurrence of HCC. Our aim was to find the percentage of HCC patients who developed recurrent disease post liver transplant. We also wanted to find if there was a surveillance strategy for cross sectional imaging or biochemical monitoring as part of the post liver transplant follow up in our patients (transplanted for HCC)

Method: A retrospective study was conducted on all patients who underwent liver transplant at Queen Elizabeth Hospital Birmingham for HCC between 2010 and 2017 and the factors associated with recurrence of cancer were studied in this cohort.

Results: A total of 299 patients with HCC underwent liver transplant in 8 years. 19 patients developed recurrence of HCC post-transplant (6.3%). Median time to diagnosis of recurrence was 17 months. All patients with HCC recurrence had AFP checked (median time to AFP check was 10months) and had imaging done post-transplant (average time to CT was 19months). All patients transplanted were within Milan criteria and no patient required down staging. About 75% of patients with recurrence did not have a significantly elevated AFP pre transplant.

Conclusion: Currently there has been no surveillance strategy for HCC screen/early detection of recurrence in our follow up at QEHB post liver transplant however this practice is not against UK guidelines. Interestingly the rate of HCC recurrence in our transplanted patients was lower than the international incidence which is likely due to strict adherence to Milan criteria. RETREAT score is a good prognostic marker as scores>5 are associated with poorer post Liver transplant outcomes and can potentially improve selection pre liver transplant to improve outcomes. It can also potentially have an impact on decision regarding post liver transplant immunosuppression and possible adjuvant therapies. RETREAT scores have been recommended in USA to work out a surveillance strategy. However it is difficult to justify a surveillance strategy in the absence of effective antineoplastic agent as no substantial benefit from early diagnosis of recurrence would be obtained. We cannot advocate for regular surveillance for HCC recurrence post liver transplant based on our study results. However we conclude that pre liver transplant screening for eligibility is the key to successful outcome.

Figure:
Series 1: Microvascular invasion
(X axis yes-if microvascular invasion present, no-if microvascular invasion absent
Y axis-number of patients)



PO-148
Systemic treatments with tyrosine kinase inhibitors and platinum-based chemotherapy in patients with unresectable or metastatic hepato-cholangiocarcinoma

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Backgrounds and aims: Although there is currently no validated systemic therapy for unresectable hepato-cholangiocarcinoma (cHCC-CCA), tyrosine kinase inhibitors and platinum-based chemotherapy are frequently used in clinical practice. Our study has aim to describe the effectiveness of first-line systemic treatments in patients with cHCC-CCA.

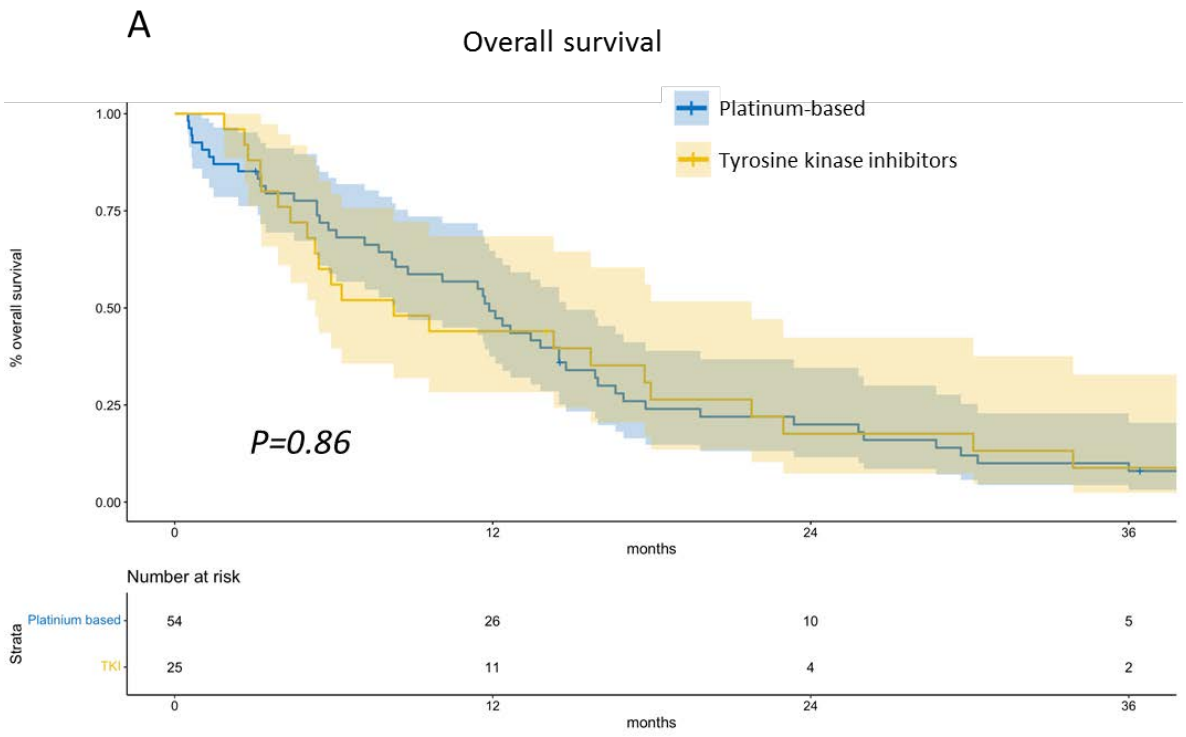
Methods: Patients with histological diagnosis of unresectable or metastatic cHCC-CCA confirmed by a centralized review (WHO classification 2019) who received systemic treatment from 2009 to 2020 were included retrospectively in 11 centres. The outcomes of patients with cHCC-CCA were compared with patients with hepatocellular carcinoma (HCC) treated by sorafenib (n=225) and with intrahepatic cholangiocarcinoma (iCCA, n=94) treated mainly by platinum using a frailty Cox model. The efficacy of tyrosine kinase inhibitors and platinum-based chemotherapies in patients with cHCC-CCA was assessed using a doubly robust estimator.

Results: A total of 83 patients with cHCC-CCA were included and were predominantly male (72%), 67% of patients had extrahepatic metastases and 31% macrovascular tumour invasion. Compared with iCCA (n=94) and HCC (n=225), cHCC-CCA were more often developed on cirrhosis (55%) than iCCA (27%) but less frequently than HCC (88%) (p<0.001). cHCC-CCA (66%) and HCC (30%) had extrahepatic metastases less frequently than iCCA (81%) (p<0.001). Unadjusted overall survival was better in CCA (13 months) compared to cHCC-CCA (12 months) and HCC (9 months) (p=0.017). In multivariate analysis after adjustment by a Cox frailty model, patients with cHCC-CCA had the same survival than HCC and iCCA (HR=1.5; 95% CI=0.98,2.3; p=0.063). ALBI score (HR=2.15; CI05%:1.23,3.76; p=0.09), ascites (HR=3.45, CI95%:1.31,9.03; p=0.013) and tobacco (HR=2.29; CI95%:1.08,4.87; p=0.032) were independently associated with OS in patients with cHCC-CCA. Among patients with cHCCCCA, 25 patients treated with tyrosine kinase inhibitors were compared with 54 patients with platinum-based

chemotherapies. Patients treated by tyrosine kinase inhibitors had a median overall survival of 8.3 months compared to 11.9 months for patients treated with platinum-based chemotherapies (p=0.86)(Figure). After a robust double adjustment on tumour number and size, vascular invasion, ALBI, MELD and cirrhosis, treatment's regimen had no influence on overall survival (HR=0.92, 95%CI=0.27-3.15, P=0.88) and progression free survival (HR=1.24, 95%CI=0.44-3.49, P=0.67).

Conclusions: Systemic treatments with tyrosine kinase inhibitors or platinum-based chemotherapies have similar efficacy in first line of patients with unresectable/metastatic cHCC-CCA. The ALBI score predicts overall survival.

Figure:



PO-150
Involvement of E2F2-miR34a-5p axis in the metabolic dysregulation of MAFLD-related HCC

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Background and Aims: Metabolic associated fatty liver disease (MAFLD) is the most common cause of chronic liver disease in Western countries and a risk factor for hepatocellular carcinoma (HCC). E2F2 is a metabolic driver in MAFLD development and progression to HCC. A relationship between E2F2 and microRNAs (miRNAs) expression has been described in different pathologies, but not in liver disease. The aims here were: 1) to identify if liver E2F2 regulates miRNAs involved in MAFLD development and progression to HCC; 2) to investigate the metabolic relationship between E2F2 and the identified miRNAs in liver pathology.

Method: To induce MAFLD-related HCC, diethylnitrosamine (DEN) (25 mg/kg) was administrated to 14 day-old *E2f2*$^{-/-}$ and wild-type mice (WT), which were fed a high-fat diet (HFD) until sacrificed at 9 months. Chow diet-fed (CD) 3 month-old *E2f2*$^{-/-}$ and WT mice were also used. E2F2 was specifically upregulated in liver with adeno-associated viruses-serotype 8. miRNA sequencing and transcriptome analysis was performed in 9 month-old DEN-HFD WT and *E2f2*$^{-/-}$ mice livers. Selected differentially expressed liver miRNA candidates and genes were further validated by RT-qPCR analysis. Metabolic fluxes and lipid content were analysed.

Results: The results obtained from miRNA sequencing and later validation by qPCR showed that in 9 month-old DEN-HFD *E2f2*$^{-/-}$ mice, resistant to MAFLD-related HCC, expression of miR-122-5p was upregulated while that of miR34a-5p, miR155-5p and miR146a-5p were downregulated. Among all, miR34a-5p was the only one decreased in CD-fed *E2f2*$^{-/-}$ while increased when E2F2 was specifically overexpressed in liver, as compared to the corresponding controls. The crosschecked analysis between the upregulated transcriptome genes underlying the resistance to develop MAFLD-related HCC in *E2f2*$^{-/-}$ mice and the miR34a-5p predicted interacting genes showed, among others, the enrichment in “Bile acid transport” and “lipid metabolism” biological pathways. Given the relevance of cholestasis in liver disease, the expression and protein levels of HNF4, a critical regulator of bile metabolism, and of *Etnk2* and *Chpt1*, modulators of the synthesis of phosphatidylethanolamine (PE) and phosphatidylcholine (PC), required for bile secretion, were analysed. The results showed higher levels of the regulators of bile metabolism in 9 month-old DEN-HFD *E2f2*$^{-/-}$ mice than in DEN-HFD WT mice together with increased PE and PC synthesis which led to higher PE, but not PC liver content, suggesting increased elimination into bile.

Conclusion: miR34a-5p is a target of E2F2 in MAFLD-related HCC. The results suggest that the E2F2-miR34a-5p axis induce inefficient bile secretion during MAFLD development and progression to HCC promoting development of the disease.

PO-154
Association between metabolic disorders and biliary tract cancer: impact of an emergent risk factor in a real-world cohort.

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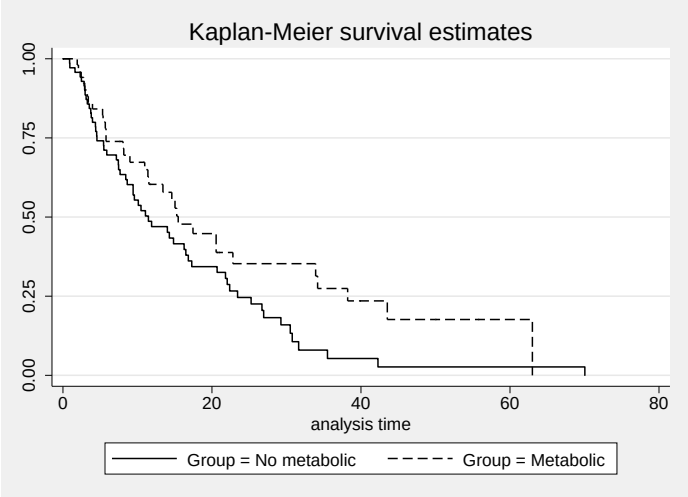
Background and Aims: The incidence of cholangiocarcinoma (CCA) has been increasing recently. Concomitant increases in metabolic syndrome across CCA cohorts may indicate a causative relation between metabolic comorbidities and CCA. However, there is a paucity of data supporting this assumption. We aimed to describe the prevalence of metabolic disorders in a CCA cohort and report clinical features and outcomes.

Method: Cohort study including patients with CCA. Demographics, medical history, treatments and outcomes were collected. Patients were divided into two groups: (1) past medical history of diabetes or/and overweight/obesity ("metabolic disorder group") and (2) without any of these features ("no-metabolic disorder group").

Results: We included 122 patients. Intrahepatic CCA (n=48; 39%) was the predominant site. Thirty-six (29.5%) patients had overweight/obesity and 24 (19.7%) had diabetes. Previous hepatopathies or biliary diseases accounted for 14.7%. Twenty-nine (23.8%) patients had resectable disease and were treated with upfront surgery. Recurrence occurred in 22 patients with a median time-to-recurrence of 14 months (95%CI 8.2-19.8). A total of 104 (85.2%) received chemotherapy for advanced/recurrent disease, mainly cisplatin-gencitabine (94.2%). The overall survival of the entire cohort was 14.3 months (95% CI: 10.1-17.3). ECOG-PS 0 (p<0.0001), resectable disease (p=0.018); well-differentiated histology (p=0.004) and absence of vascular invasion (p=0.048) were independently associated with better survival. No significant differences in baseline characteristics were found between the "metabolic disorder" (n=52) and the "no-metabolic disorder" group (n=70). The "metabolic disorder" group had a median survival of 15.5 months (95%CI 10.9-33.9) versus 11.5 months (95%CI 8.4-16.5) in the "no-metabolic disorder" group (adjusted HR: 1.10; 95%CI 0.62-1.94). Patients with resectable disease in the "metabolic-group" were less likely to presented recurrence (p=0.039) and had a significantly better survival than patients in the "no-metabolic" group (43.4 [95%CI 33.9-NR] versus 21.8 months [95%CI 8.6-26.9]; HR=0.23, 95%CI 0.06-0.86).

Conclusion: Metabolic disorders are frequent among CCA patients and its relationship, although attributable, is not totally understood. Underlying metabolic comorbidities may be associated with prognosis in CCA. There is a need to explore the mechanism that drives CCA carcinogenesis in a metabolic background.

Figure:



Kaplan meier curves showing the overall survival according to subgroup with metabplic disorders vs no metabolic disorders.

PO-159
Epidemiology of hepatocellular carcinoma in Portugal

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Background and Aims: There is scarce data on the burden of hepatocellular carcinoma (HCC) in Portugal. We aim to characterize the recent epidemiology of HCC in Portugal.

Method: We evaluated HCC-related hospital admissions and mortality in Portugal between 2010 and 2017. We analysed all hospital admissions from patients with HCC in public hospitals (data from Portuguese Health System's Central Administration) and mortality due to HCC (data from National Statistics Institute) – coded with 155.0 (International Classification of Diseases – ICD-9-CM) or C22.0 (ICD-10). Additional analyses were performed to evaluate longitudinal trends and regional differences.

Results: Between 2010 and 2017, there were 20,704 hospital admissions of patients with HCC, ranging annually from 2,086 in 2010 to 2,989 in 2015. In-hospital mortality rate during admission was 16.7%.

Considering individual patients admitted with HCC, the number of patients admitted annually varied from 1,046 in 2010 to 1,560 in 2017. 7,739 individual patients were admitted during the 8-year period: 80.1% males and with mean age 66 ±12.2 years.

In the whole country, both in-hospital and ambulatory, the annual number of deaths ranged from 471 in 2010 to 620 in 2017 (4,316 deaths during the 8 years). Potential years of life lost ranged annually from 2477 in 2010 to 3838 in 2017.

Annual number of admissions, admitted patients, mortality and potential years of life lost are detailed in the table.

The mean annual mortality rate due to HCC, considering the 8-year period, was 5.2/100,000 inhabitants (9.0/100,000 inhabitants among males and 1.8/100,000 inhabitants among females). The highest regional mortality rate was observed in the Lisbon metropolitan area (6.3/100,000 inhabitants overall, 11.2/100,000 inhabitants among males and 2.0/100,000 inhabitants among females).

Conclusion: The burden of HCC in Portugal is high and had an increasing trend between 2010 and 2017.

Figure:

	2010	2011	2012	2013	2014	2015	2016	2017	Longitudinal trend 2010-2017 (p-value)
Hospital admissions (n)	2086	2600	2425	2641	2694	2989	2793	2476	(p = 0.125)
Admitted patients (n)	1046	1205	1220	1376	1371	1511	1560	1410	Increasing (p = 0.003)
Deaths (n)	461	557	558	496	535	536	553	620	(p = 0.073)
Potential years of life lost (n)	2477	3215	3340	2955	3228	3005	3013	3838	(p = 0.126)

PO-160
CXCR2 inhibition sensitises to anti-PD1 therapy in NASH-HCC

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Background and Aims: Non-alcoholic steatohepatitis (NASH) is increasingly a major underlying cause of hepatocellular carcinoma (HCC). Immunotherapy offers great promise for HCC therapy; however, recent published data suggests that NASH is the cause of immune changes that negatively impacts on the efficacy of conventional immune checkpoint inhibition (ICI). Here we aimed to sensitise NASH-HCC to anti-PD1 therapy by targeting neutrophils using a CXCR2 small molecule inhibitor (AstraZeneca - AZD5069).

Method: Neutrophil infiltration was characterised in multiple models of murine HCC and in human patient biopsies. Late-stage intervention with anti-PD1 and/or AZD5069 was performed in two NASH-HCC models (orthotopic and autochthonous). The tumour microenvironment was characterised by a combination of immunohistochemistry, flow cytometry and RNA-seq.

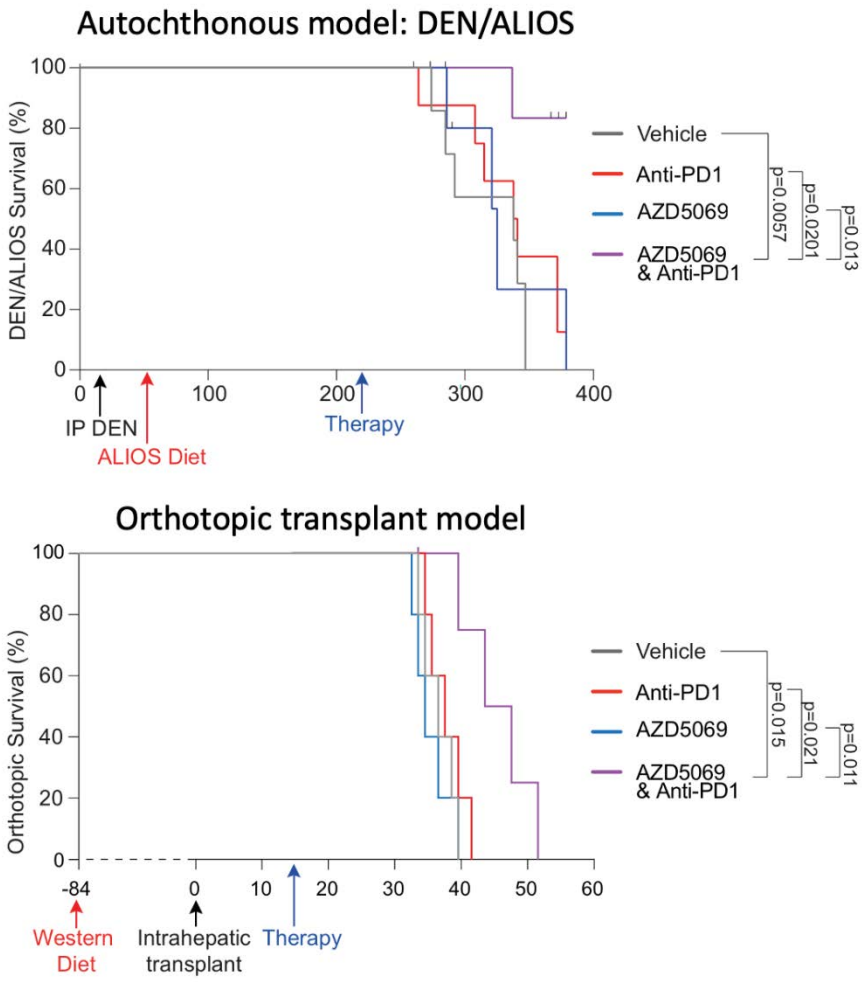
Results: $CXCR2^+$ neutrophils were found to be highly represented in both murine and human NASH-HCC.

In models of NASH-HCC lacking response to ICI, the combination of AZD5069 with anti-PD1 effectively suppressed tumour burden and extended survival (Fig 1). The combination therapy increased intratumoural $CD103^+XCR1^+$ dendritic cells and CD8+ T cells that are associated with anti-tumoural immunity. The therapeutic effect was lost upon genetic impairment of dendritic cells or antibody mediated depletion of $CD8^+$ T cells. Combination therapy resulted in an unexpected increase in tumour-associated neutrophils (TANs). These TANs were found to be proliferative and by the use of image mass cytometry to be located within immunological hubs in direct contact antigen presenting cells and $CD8^+$ T cells. These immune hubs were enriched for the cytotoxic anti-tumoural protease granzyme B which was found at increased levels in tumours co-treated with CXCR2 antagonist and anti-PD1 compared with monotherapy groups.

TANs in combination-treated tumours displayed a switch from a pro-tumour to anti-tumour progenitor-like neutrophil phenotype, closing resembling a recently characterised acute-inflammatory immature-$Ly6G^{Int}$ neutrophil population isolated from lipopolysaccharide-(LPS)-treated mice. Intravenous infusion of these neutrophils into orthotopic tumour bearing mice, sensitised to anti-PD1 therapy, promoting dendritic cell and $CD8^+$ T cell recruitment and activation.

Conclusion: CXCR2-inhibition induces multi-cellular reprogramming of the tumour immune microenvironment that promotes ICI treatment of HCC in the context of NASH.

Figure:


Autochthonous model: DEN/ALIOS
DEN/ALIOS Survival (%)
100
80
60
40
20
0
0
100
200
300
400
IP DEN
ALIOS Diet
Therapy
Vehicle
Anti-PD1
AZD5069
AZD5069
& Anti-PD1
p=0.0057
p=0.0201
p=0.013
Orthotopic transplant model
Orthotopic Survival (%)
100
80
60
40
20
0
-84
0
10
20
30
40
50
60
Western
Diet
Intrahepatic
transplant
Therapy
Vehicle
Anti-PD1
AZD5069
AZD5069
& Anti-PD1
p=0.015
p=0.021
p=0.011

PO-161
Opposite roles for PD-1 expressing tissue resident and exhausted T cells in Hepatocellular Carcinoma

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Background and Aims: PD-1 expression by CD8 T cells in the tumour microenvironment is frequently used as an exhaustion marker. However, PD-1 expression is not restricted to exhausted T cells (TEX), and can also be expressed by effector or tissue resident T cells (TRM) or, in the CD4 compartment, regulatory T cells or follicular helper T cells. Here, we aimed to understand the role of PD-1 expressing T cells in the tumour micronenvironment of HCC patients by using a deep immune profiling approach dissecting CD8 and CD4 populations with PD-1 expression.

Method: Mass cytometry was used for comprehensive profiling of matched tumour and peripheral blood samples from HCC patients of different etiologies. Single-cell- and population based transcriptome profiles from published datasets of HCC resections were in silico assessed for signatures of T cell exhaustion and residency and their link to patient survival. Highly multiplexed imaging mass cytometry was used to assess the spatial role of TEX and TRM populations. The analysis was expanded to a cohort of patients consecutively treated with anti-PD-1 immunotherapy.

Results: We observed a strong enrichment of PD-1 expressing T cells in the tumour microenvironment. However, the PD-1+ immune infiltrate was heterogeneous for expression of other exhaustion or tissue resident markers. Interestingly, a high proportion of PD-1+ T cells expressing additional tissue resident markers was associated with prolonged progression free survival, more dense immune infiltration and viral etiology while a high proportion of more severely exhausted T cells was associated with poor survival, metabolic liver disease etiology and immune dysfunction. A higher enrichment of TRM over TEX also was also positively associated with disease control in checkpoint-treated patients.

Conclusion: These data demonstrate the clinical relevance of the balance of exhausted and resident memory T cells, which can both express PD-1, in HCC patients. Precise determination of TRM and TEX cells in patient biopsies may help in rational patient selection for immunotherapies.

PO-163
The NADPH oxidase NOX4 regulates redox and metabolic homeostasis in hepatocellular carcinoma

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Background and Aims: The NADPH oxidase (NOX) family has emerged in the last years as an important source of reactive oxygen species in signal transduction. NOX4 isoform has been implicated in a variety of physiological and pathological processes. Our research group found that silencing NOX4 expression in hepatocellular carcinoma (HCC) cells increases their proliferative capacity in vitro and enhances their tumourigenic potential in mice, resulting in earlier onset of tumour formation and increase in tumour size. NOX4 also regulated cellular processes that occur later in progression and that favor tumour metastasis, such as migration and invasion. Here, we aim to determine the molecular mechanisms regulated by NOX4 in HCC cells that could explain its tumour suppressor functions.

Method: Proteomic, transcriptomic, metabolomic and functional analyses in HCC cells with NOX4 silenced or overexpressed.

Results: The proteomic analysis, comparing HCC control cells with cells where NOX4 had been silenced or overexpressed, allowed the identification of changes in the levels of proteins whose expression is under the control of MYC and NRF2. Importantly, the activation of both transcription factors inversely correlated with NOX4 levels. In addition, silencing MYC reverted the increase in cell proliferation and migration mediated by NOX4. Moreover, silencing NOX4 induced changes in the amount and dynamics of mitochondria, increased protein levels of ETC machinery, and ATP levels, mediated by MYC activation. Furthermore, silencing NOX4 levels in HCC cells increased both glycolysis and OXPHOS pathways, while NOX4 overexpression showed opposite effects. Transcriptomic and metabolomic analyses indicated that NOX4 could be also regulating fatty acid metabolism. Importantly, the inverse correlation between NOX4 and MYC, as well as with some key metabolic enzymes was observed in human HCC samples. Furthermore, NOX4 loss altered the cellular redox balance that produced increase in NRF2 activity, which was responsible for MYC activation. Low NOX4 levels induced the expression of NOX1/NOX2, which contributed to oxidative stress and consequently to NRF2 activation. Analogously, NOX4 silencing reduces proteasomal activity, which may impact on NRF2 degradation, contributing to its constitutive activation. Finally, NOX4 catalytic activity was required for some of the effects observed.

Conclusion: Liver tumour suppressor functions of NOX4 could be explained through its role in regulating redox and metabolic cell homeostasis in a NRF2/MYC dependent fashion.
Funding: Grants to IF from Ministry of Science and Innovation (cofounded by FEDER funds/Development Fund-a way to build Europe): SAF2015-64149-R, RTI2018-094079-B-100 and RED2018-102576-T. Fellowship to IP-H: BES-2016-077564. The CIBEREHD, funded by the ISCIII, Spain. CERCA Programme/Generalitat de Catalunya for institutional support.

PO-170
P53 and VEGF immunoexpression are predictive biomarkers of Sorafenib efficacy in an experimental model of NASH-related HCC

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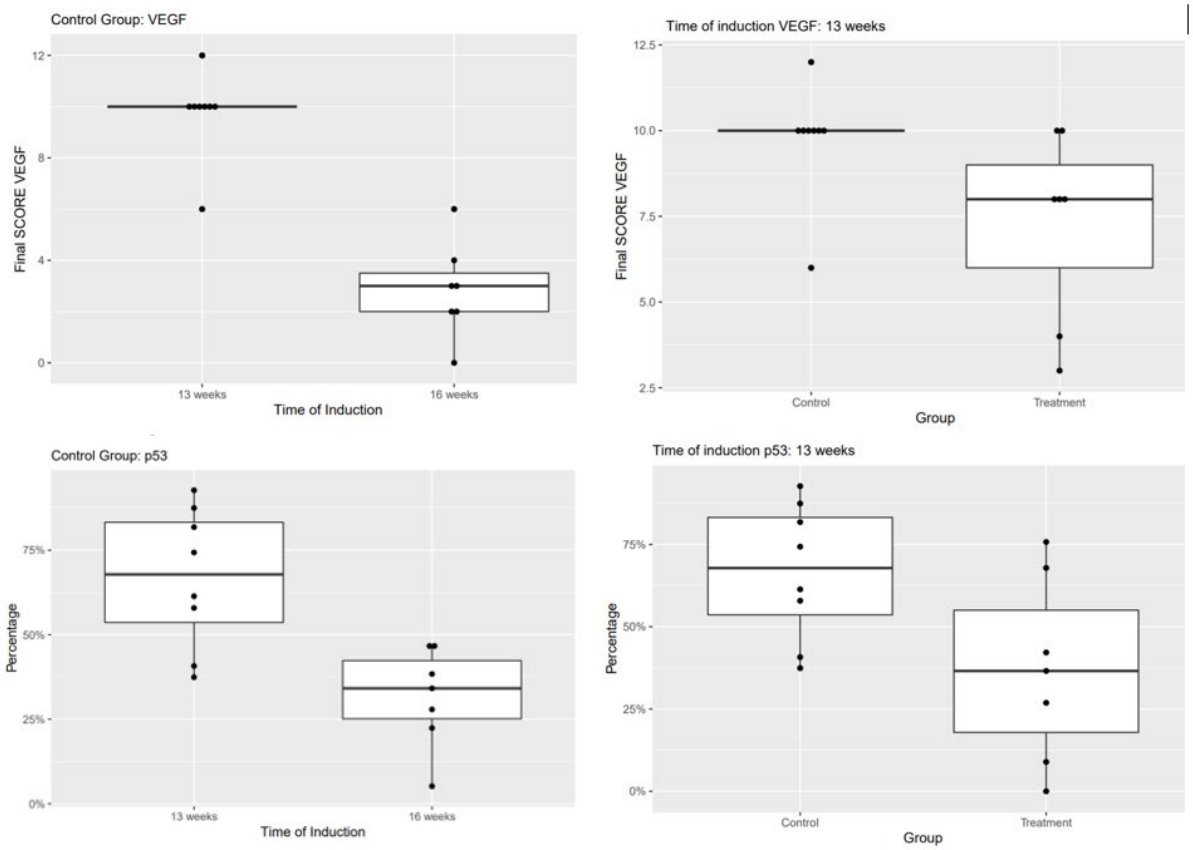
Background and Aims: The efficacy of systemic therapy for Hepatocellular carcinoma related to nonalcoholic steatohepatitis (NASH-HCC) are little known. In this study we evaluated the effects of sorafenib through the immunoexpression of molecular markers (cell proliferation and angiogenesis) in a NASH-related HCC model.

Method: 40 male Sprague-Dawley rats were subjected to NASH-HCC model through the combination of a high-fat and choline deficient diet and diethylnistrosamide (100 mg/L) in the drinking water. After 13 or 16 weeks of induction of carcinogenesis, the animals were randomly divided into 4 experimental groups: 2 control groups (vehicle) and 2 treatment groups sorafenib (5mg/kg/day) by gavage. After 2 weeks of treatment the animals were euthanized and tissue samples from liver nodules were collected for histopathological and immunohistochemical analysis of HEP-PAR-1, glutamine-synthetase, VEGF, survivin, β-catenin and p53. The histopathology of liver tumours and the HEP-PAR-1 and glutamine-synthetase immunoexpression were used to HCC diagnosis. A semi-quantitative score was used for VEGF, survivin and β-catenin analysis by the percentage of the area and the intensity of the staining in the selected neoplastic nodules. The intensity was scored as 1 (no staining), 2 (light), 3 (moderate); and 4 (strong). The area was scored based on the percentage of neoplastic cells stained: 0 (no staining); 1 (0-5%); 2 (6-25%); 3 (26-50%); 4 (51-75%) and 5 (76-100%). The final score was obtained from the intensity score multiplied by the area score. For p53, the percentage of positive cells were determined in 1,000 neoplastic cells. Results were processed by Wilcoxon´s test and/or student´s t-test.

Results: Both hepatocarcinogenesis time induction were successful in generating HCCs and showed a loss of reactivity to HEP-PAR-1 and an accumulation of glutamine-synthetase in the neoplastic cells. Sorafenib-treated animals showed a decreased immunoexpression of VEGF and p53 at 13 weeks when compared to control group ($p=0.03$; $p=0.04$, respectively). No significant difference in β-catenin and survivin immunoexpression was observed. The analysis to evaluate the tumoural progression showed a significant decrease of VEGF and p53 immunoexpression in the control group of 16 weeks when compared to the 13 weeks group ($p<0.01$).

Conclusion: 1. The reduced immunoexpression of p53 and VEGF by sorafenib indicates that these are promisor biomarkers for treatment response assessment in NASH-HCC. **2.** The weak staining pattern of survivin and β-catenin observed in NASH-HCC in this model, did not show to be good indicators of Sorafenib efficacy response **3.** The decrease in p53 and VEGF immunoexpression observed in the control group (16-weeks) when compared to the 13-weeks may represent a shift in the onset moment of HCC's biology and also in the molecular pathway's activity involved in tumour progression.

Figure:


Control Group: VEGF
Final SCORE VEGF
12
8
4
0
13 weeks
16 weeks
Time of Induction
Time of induction VEGF: 13 weeks
Final SCORE VEGF
12.5
10.0
7.5
5.0
2.5
Control
Treatment
Group
Control Group: p53
Percentage
75%
50%
25%
0%
13 weeks
16 weeks
Time of Induction
Time of induction p53: 13 weeks
Percentage
75%
50%
25%
0%
Control
Treatment
Group

PO-175
A retrospective real-world study of transarterial gemcitabine-based chemoembolization plus lenvatinib with or without a PD-1 inhibitor for unresectable intrahepatic cholangiocarcinoma

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Background and Aims: Most patients with intrahepatic cholangiocarcinoma (ICC) present with unresectable locally advanced or metastatic disease, and outcomes with standard first-line gemcitabine-based chemotherapy remain poor. Transarterial chemoembolization (TACE) has demonstrated efficacy in advanced ICC, and the combination of lenvatinib and programmed death-1 (PD-1) inhibitors has shown encouraging results. However, the efficacy of TACE combined with lenvatinib with/without a PD-1 inhibitor is unknown.

Method: This was a retrospective study of patients with unresectable ICC treated at Fudan University Shanghai Cancer Center between Jan 2017 and Aug 2021. The inclusion criteria were as follows: treatment with gemcitabine-based TACE combined with lenvatinib (8 mg orally QD) with/without a PD-1 inhibitor (camrelizumab or sintilimab 200 mg Q3W via intravenous infusion) in any line of treatment, ECOG PS 0-1, Child-Pugh A-B. Overall survival (OS) and progression free survival (PFS) were evaluated by the Kaplan-Meier method, and response was assessed according to mRECIST 1.0. Risk factors associated with OS were assessed using univariate and multivariate Cox regression analysis. The cut-off date for the analysis was 8 August 2021.

Results: In total, 32 patients with pathologically confirmed ICC were included (median age 58.5 years [range 39-78 years]; 20 males and 12 females). The median follow up time was 19.8 months (range 1.8-37.8 months). A total of 20 patients received TACE and lenvatinib (group TL), and 12 patients received TACE and lenvatinib plus a PD-1 inhibitor (group TLP, camrelizumab or sintilimab). Among all patients, the median OS was 25.3 months (95% confidence interval [CI] 18.5-32.1). The median PFS was 7.3 months (95% CI 4.9-9.7). The median OS in the TL group and TLP group was 22.4 vs 27.3 months, respectively (p=0.695). In the regression analysis, independent favorable prognostic factors for OS were hepatitis B/C (hazard ratio [HR] 0.63, 95% CI 0.009-0.463; p=0.038) and previous ICC resection (HR 0.007; 95% CI 0.009-0.463; p=0.007). There were no treatment-related deaths. The occurrence of any-grade adverse events (AEs) was 81%. The most common AEs were decreased appetite (11 [34.3%]), hypertension (10 [31.2%]), and diarrhea (9[28.1%]).

Conclusion: TACE combined with lenvatinib with/without a PD-1 inhibitor provided promising outcomes for patients with unresectable ICC. Prospective, large sample size trials are needed for further investigation and validation of this treatment approach.

PO-176
Factors associated with the radiological response of atezolizumab plus bevacizumab for unresectable hepatocellular carcinoma

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Background and Aims: Atezolizumab plus bevacizumab (Atezo+Bev) is recommended as 1st-line therapy for unresectable hepatocellular carcinoma (u-HCC). We investigated factors associated with the radiological response of Atezo+Bev for u-HCC and the relationship between AFP and time to progression (TTP).

Method: A total of 81 patients who received Atezo+Bev at our hospital between October 2020 and November 2021 was enrolled. Radiological evaluation was performed at baseline, 6-8 weeks after Atezo+Bev administration, and every 6-8 weeks thereafter. The best response was evaluated by the Response Evaluation Criteria in Solid Tumours (RECIST) 1.1.

Results: The patients received Atezo+Bev as 1st-line (n = 45), 2nd-line (n = 19), 3rd-line (n = 7), 4th-line (n = 6), 5th-line (n = 3) and 6th-line therapy (n = 1). In 66 patients who performed radiological evaluation, the objective response rate (ORR) and disease control rate (DCR) were 25.8% and 74.2%, respectively. No Bev reduction or discontinuation owing to adverse events (AEs) within 12 weeks was a significant ORR-related factor (p = 0.02). Etiology, BCLC stage, and ALBI grade were not associated with ORR. In 12 patients who reduced or discontinued Bev, the rate of patients who received Atezo+Bev as later-line therapy was higher than in 54 patients who did not require Bev dose reduction (83.3% vs. 35.2%, p = 0.003), and baseline urine protein creatinine ratio was higher (median 0.13 vs. 0.07, p = 0.007). The median TTP was significantly longer in 37 patients receiving as 1st-line therapy than in 29 patients receiving as later-line therapy (not reached vs. 3.8 months, p < 0.001). The median TTP was significantly longer in 54 patients treated with Bev full-dose than in 12 patients who required Bev dose reduction or discontinuation (7.0 months vs. 3.3 months, p = 0.01). DCR-related factors were etiology (viral) and baseline neutrophil to lymphocyte ratio (NLR) < 3.6 (p = 0.005, p = 0.008). No Bev reduction or discontinuation owing to AEs within 12 weeks, BCLC stage and ALBI grade were not associated with DCR. As a predictor of DCR, the positive predictive value of AFP response (reduction ≥ 20% from baseline) at 6 weeks was 96.2%. Twenty-six patients with AFP response at 6 weeks had a significantly longer TTP than 40 patients without AFP response (median 8.8 months vs. 3.8 months, p = 0.001). The median TTP was significantly shorter in 27 patients with AFP progression (increase ≥ 10% from baseline) at 6 weeks than in 39 patients without AFP progression (3.8 months vs. 8.7 months, p = 0.005).

Conclusion: Patients who received Atezo+Bev as 1st-line therapy had better clinical outcomes than those treated as later-line therapy. Bev dose reduction or discontinuation owing to AEs within 12 weeks, etiology, and baseline NLR < 3.6 may be associated with radiological response. Changes in AFP at 6 weeks could be a predictor of disease progression.

PO-177
Cholangiocarcinoma-on-chip: a 3D liver tumour model

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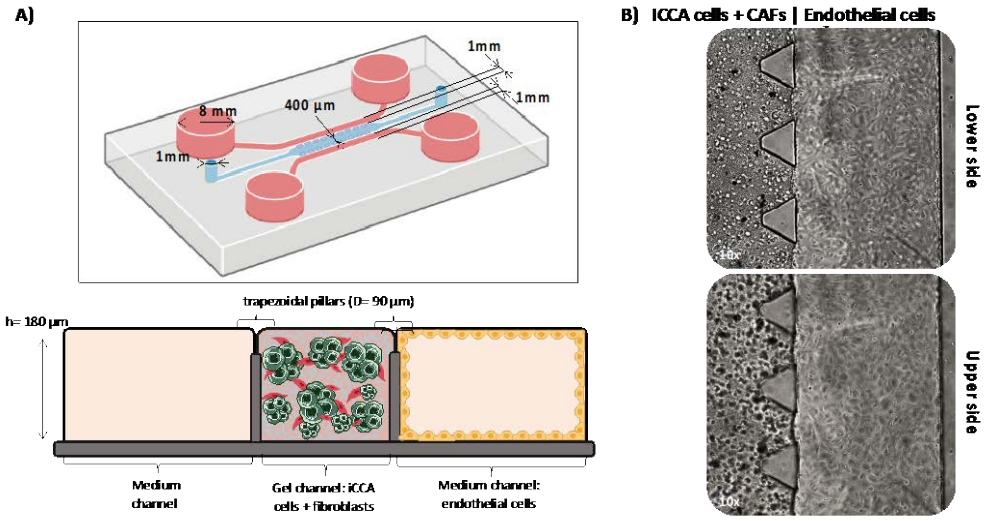
Background and Aims: Intrahepatic cholangiocarcinoma (iCCA) is a deadly cancer of biliary epithelium with very limited therapeutic options. This highlights the importance of decipher iCCA mechanisms for effective therapeutic strategies. Nevertheless, *in-vivo* cellular interactions complexity has hindered an effective recapitulation of *in-vitro* human milieu through 2D culture systems. We aim to develop an *in-vitro* 3D microfluidic device with patient-specific co-cultures of the main cells involved in iCCA.

Method: Primary iCCA cells were isolated from patients surgically resected at the Hepatobiliary Surgery Department, Humanitas Clinical Institute. A PDMS microfluidic device, composed by three adjacent microchannels separated by pillars and with independent accesses, was fabricated at Polytechnic of Milan.

Results: Firstly, proliferation assays, morphology assessments and RT-PCR were performed for three cell types to verify the phenotype retaining with an *ad-hoc* medium composition. After medium optimization, to recapitulate iCCA microenvironment, iCCA cells and primary fibroblasts were co-cultured in the central channel embedded in an optimized fibrin/collagen hydrogel, displaying an high cell viability and 3D organization after 96h in culture. Endothelial cells were seeded in one lateral channel forming a tubular vessel, corroborated by fluorescence microscopy with specific antibodies. Dextran diffusion assays and COMSOL simulations were performed to assess the hydrogel diffusion ability to molecules and the endothelial barrier functional integrity within the chip.

Conclusion: Our results showed that we were able to recreate a reliable iCCA microenvironment in a 3D microfluidic device. This system will make it possible to elucidate the biological mechanisms involved in iCCA progression and may provide an efficient clinical tool for personalized drug testing.

Figure 1: Cholangiocarcinoma-on-chip



PO-179
Altered glutamine metabolism in intrahepatic cholangiocarcinoma: biological effects of glutaminase inhibitor

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Background and Aims: Intrahepatic cholangiocarcinoma (iCCA) is a deadly cancer arising from biliary epithelial cells (BECs) lining the biliary tree. iCCA is a highly chemoresistant tumour and pharmacological therapies are generally unsuccessful. Furthermore, due to the complexity of the *in-vivo* cellular interactions, metabolic activation pathways are largely unknown. We herein aim to elucidate the metabolic asset of BECs and iCCA cells.

Method: BECS and iCCA cells were isolated from patients resected at the Division of Hepatobiliary and General Surgery, Humanitas Clinical Institute. BECs and iCCA surnantants were analysed by using mass spectrometry-based untargeted and targeted metabolomic approaches. RNA-seq and Reverse transcriptase-polymerase chain reaction (RT-PCR) analyses were performed to identify altered metabolic pathways in iCCA cells. Moreover, iCCA cell proliferation was assessed at different time points post glutaminase-1 (GLS-1) inhibition (CB-839) and immunofluorescence staining for mitochondria was performed.

Results: iCCA cells were characterized by enhanced mitochondrial activity compared to BECs, resulting in an increased glutamine and glucose uptake. RNA-seq analysis revealed several altered metabolic pathways in iCCA cells attributed to the Warburg effect and glutamine metabolism. To explore the importance of glutamine metabolism, we further evaluated the mRNA expression of ASCT1 and ASCT2, two glutamine transporters, and GLS1, resulted to be significantly upregulated in iCCA cells. CB-839 treatment revealed that GLS1-high iCCA cells were more susceptible to GLS-1 inhibition compared to GLS1-low cells, along with changing in mitochondrial morphology.

Conclusion: Experimental data suggest an impairment of mitochondrial activity of iCCA cells. Resensitizing iCCA cells to metabolic treatments could make them more susceptible to cytotoxic drugs, opening new possibility to improve the outcomes of the iCCA patients.

PO-181
The development of ex vivo human models of hepatocellular carcinoma in precision cut liver slices for high throughput screening of anti-cancer therapies

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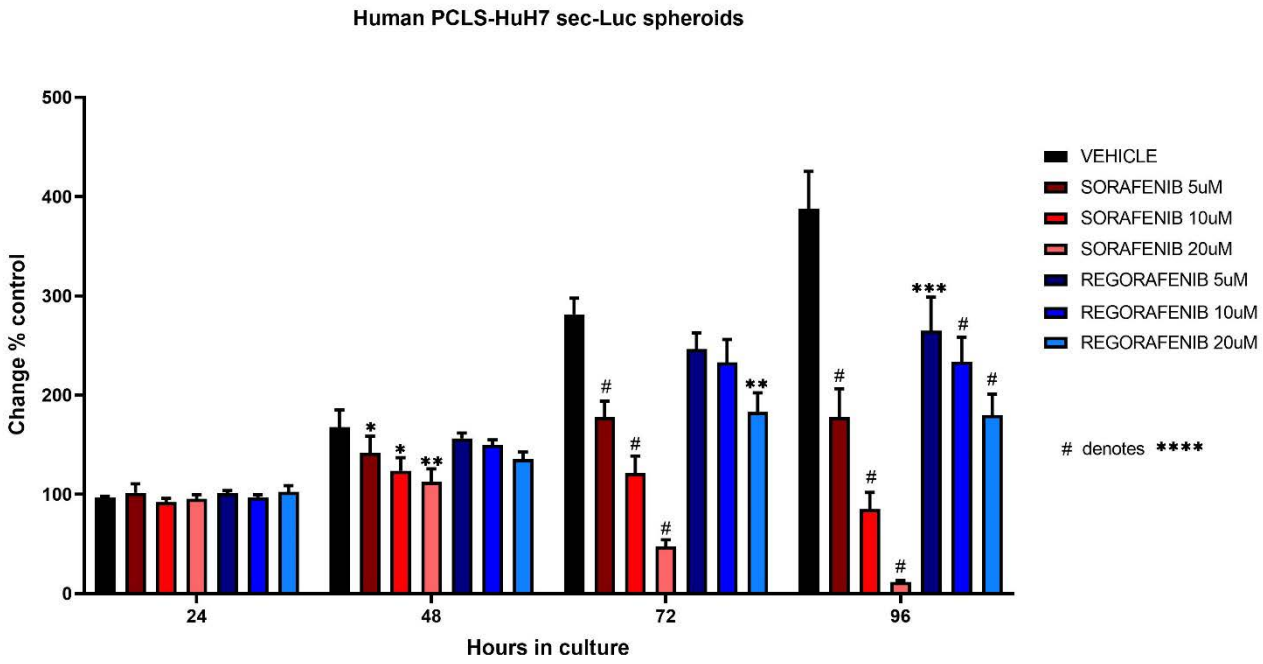
Background and Aims: Liver cancer is one of the largest contributors to cancer-related deaths worldwide, with hepatocellular carcinoma (HCC) accounting for the majority of cases. Recent advances in medical therapies offer the hope of survival beyond a few months, but only for a minority of patients. It is therefore vital that realistic HCC models are developed, bridging the gap between preclinical experimental techniques and human disease, to provide valuable insight into disease pathogenesis and drug discovery. Here we aim to develop reproducible, human models of HCC in precision cut liver slices (PCLS), combining either HCC spheroids or bio-printed cells with human liver tissue in order to recapitulate an *in situ* tumour encapsulated within the liver. These models would then be utilised for high throughput testing of novel anti-cancer mono or combination therapies.

Method: Spheroids were generated from HuH-7 cells expressing either tdTomato or secreted luciferase. PCLS were generated from human liver tissue or C57BL/6J mice. Spheroids were implanted on human PCLS and then the HCC-PCLS were cultured either with or without sorafenib or regorafenib (20 uM – 2.5 uM) for a further 4 days. Spheroid growth was assessed by measuring red fluorescent protein (RFP) levels, or tracked longitudinally via luciferase secreted into the culture media. To create a HCC-stromal structure, Hep-53.4 cells were bio-printed with 3T3 fibroblasts onto murine PCLS using reactive jet impingement (ReJI) technology. PCLS were cultured in a patented bioreactor platform.

Results: HuH-7 tdTomato spheroids implanted on human PCLS were imaged daily, confirming engraftment of the spheroids on the tissue. Multiphoton imaging confirmed that HuH-7 tdTomato spheroids completely invaded the 250 µm thick PCLS. Quantification of RFP levels in PCLS 24 and 96 hours post spheroid engraftment confirmed that the spheroids proliferate within the tissue. RFP levels were significantly decreased following treatment of the PCLS with 10 uM and 20 uM sorafenib. Similarly, 5 uM – 20 uM sorafenib and regorafenib promoted a dose dependent and significant decrease in the levels of luciferase secreted into the culture media compared to the control group (Figure 1), while tissue viability was maintained. Multiphoton imaging confirmed that ReJI bio-printed Hep-53.4 cells invade the PCLS, particularly following modification of the bio-ink to contain 3T3 fibroblasts and growth factors.

Conclusion: Initial results present both the spheroid-PCLS and ReJI-PCLS models as platforms capable of identifying novel HCC therapies, accounting for the important contributions of the surrounding liver. The capability to manipulate PCLS to mimic various states of underlying liver disease, alongside the possibility of generating spheroids from patient-derived HCC cells, present the model as a unique tool for personalised medicine.

Figure:


Human PCLS-HuH7 sec-Luc spheroids
Change % control
500
400
300
200
100
0
24
48
72
96
Hours in culture
VEHICLE
SORAFENIB 5uM
SORAFENIB 10uM
SORAFENIB 20uM
REGORAFENIB 5uM
REGORAFENIB 10uM
REGORAFENIB 20uM
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PO-190
Role of ABC drug efflux pumps in the resistance of hepatoblastoma to conventional chemotherapy

Candela Cives-Losada[1], Maitane Asensio[1 2], Elisa Lozano[1 2], Ana Peleteiro Vigil[1], Anabel Sanchez-Martin[1], Juan Carrillo[3], Oscar Briz[1 2], Stefano Cairo[4], Oliver Poetz[5], Helen S. Hammer[5], Carolina Armengol[2 3], Jordi Bruix[2 6], Jose Marin[1 2], Rocio IR Macias[1 2]
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Background and Aims: Hepatoblastoma (HB) is the most common liver cancer in children. Despite the relative success rate of conventional chemotherapy based on the combined administration of cisplatin and doxorubicin, approximately 20% of patients do not respond favorably and have a poor prognosis. This lack of response could be due to the presence of mechanisms of pharmacoresistance (MPRs), such as a reduction in the intracellular content of anti-tumour agents due to changes in the expression of drug transporters. Thus, we aimed to study the role of ABC drug export pumps in HB resistance to conventional chemotherapy.

Method: The expression of the most critical ABC genes involved in the resistome was analysed by RNA sequencing (RNA-seq) in 32 HB tumour and adjacent non-tumour (NT) samples from human biopsies. Results were further confirmed by RT-qPCR in 22 paired samples. Protein expression and localization of selected ABC pumps were determined by immunohistochemistry (IHC) in formalin-fixed, paraffin-embedded (FFPE) tumour samples. The expression and localization of these transporters were also determined in HB-derived cell lines (HepG2 and HuH6) by RT-qPCR, Western blot, immunofluorescence, and Triple X Proteomics (TXP) under basal conditions and after 72 h exposure to cisplatin or doxorubicin. Cell viability was determined by sulforhodamine B assay. The transport activity of ABC pumps was analysed by efflux assays using specific substrates and inhibitors measured flow cytometry.

Results: The high expression levels of ABCB1 (MDR1), ABCC1-5 (MRP1-5), and ABCG2 (BCRP), as well as their marked activity in both HuH6 and HepG2 cells, supports the role of these pumps in HB multidrug-resistant phenotype. Moreover, several of these transporters were highly expressed in HB. In particular, marked upregulation of ABCC1 (MRP1) and ABCC5 (MRP5) in the tumour as compared to adjacent NT tissue was found. The exposure of HB cells to 0.1 µM doxorubicin or 5 µM cisplatin for 72 h enhanced the resistant-phenotype of HB-derived cells, mainly by up-regulation of ABCG2 in HuH6 and ABCC3 in HepG2 cells.

Conclusion: Drug efflux through ABC pumps is involved in the lack of response of HB to conventional chemotherapy. Some of these transporters could be of interest as prognostic biomarkers and as therapeutic targets for developing sensitizing strategies to improve the outcome of HB patients.

PO-193
MiR-30e-3p plays a dual role in hepatocellular carcinoma and predicts sorafenib resistance

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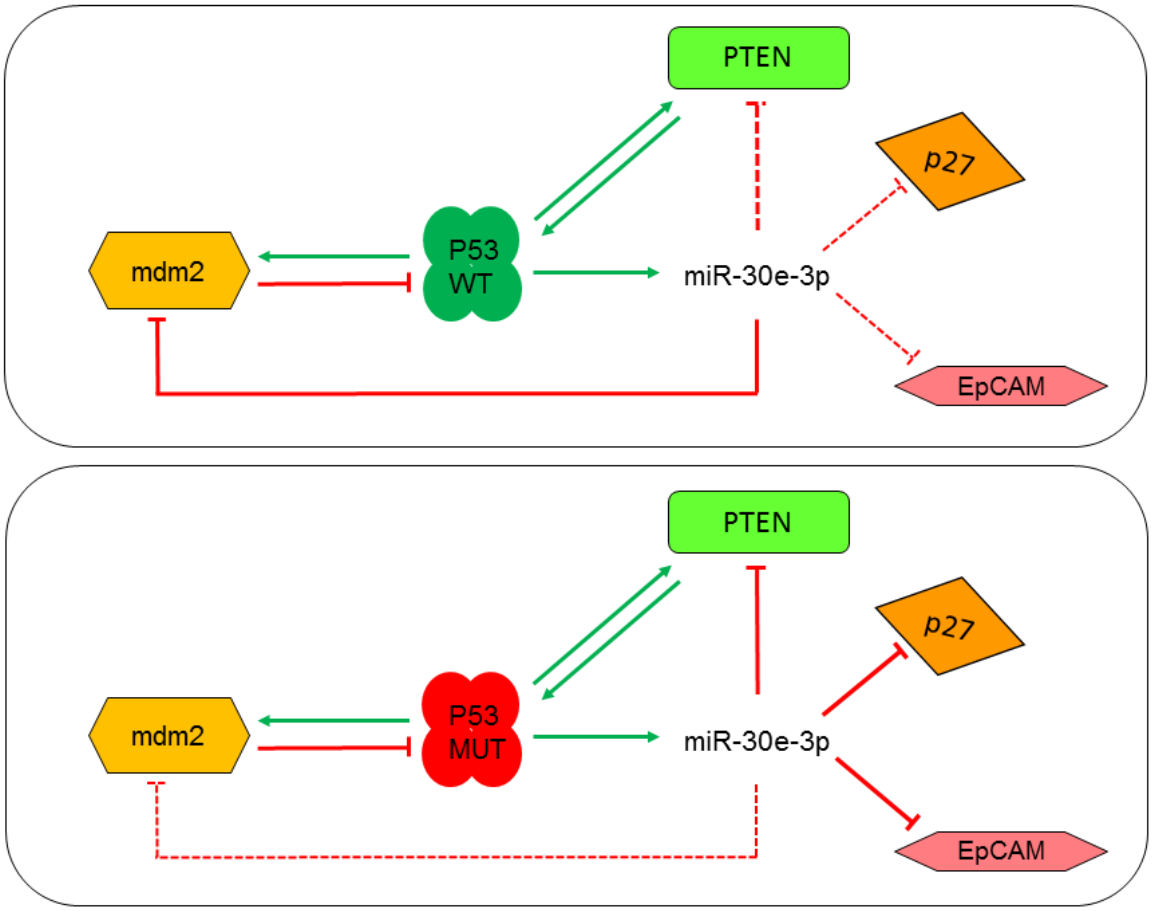
Background and Aims: Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related mortality showing an increased incidence. The molecular background of HCC is highly heterogeneous. Genomic and molecular signatures have been associated with tumour aggressiveness and prognosis, yet no validated biomarkers are available to guide clinicians. MicroRNAs (miRs) represent promising therapeutic targets and circulating biomarkers in HCC. The aims of this study are to investigate miR-30e-3p contribution to HCC phenotype and sorafenib response and to assay the predictive role of circulating miR-30e-3p levels in sorafenib-treated patients.

Method: Tissue and serum miR-30e levels were analysed by Real time PCR in HCC patients, DEN-HCC rats and HCC cell lines. Functional analysis and reporter assay were used to validate miR-30e-3p target genes in HCC cells. Proliferation, invasion and clonogenic assays established miR-30e-3p influence on HCC phenotype. Flow cytometry and caspase assay were used to evaluate miR-30e-3p involvement in stress conditions and sorafenib response. TP53 expression was modulated to test its role on miR-30e-3p extrusion.

Results: MiR-30e-3p was downregulated in human and rat HCCs, and its downregulation associated with TP53 mutations. TP53 contributed to miR-30e-3p transcription, while MDM2 was identified among its target genes, establishing miR-30e-3p/TP53/MDM2 feedforward loop. EpCAM, PTEN, and p27 were demonstrated as miR-30e-3p targets mediating its contribution to stemness and malignant features. TP53 mutational status was responsible for miR-30e-3p dual role in HCC cells with different molecular background. TP53 also influenced miR-30e-3p extrusion in sorafenib and doxorubicin treated cells. Tissue miR-30e-3p negatively correlates with CXCL3 chemokine in DEN-HCC rats treated with sorafenib, whereas circulating miR-30e-3p levels showed a positive correlation. In a preliminary cohort of HCC patients subjected to sorafenib treatment, increased miR-30e-3p circulating levels predicted the development of resistance.

Conclusion: In conclusion, TP53 status and molecular background dictate miR-30e-3p dual behaviour in HCC. Mdm2 targeting plays a predominant tumour suppressor function in wild-type TP53 contexts, whereas other targets mediate miR-30e-3p oncogenic role in non-functional TP53 backgrounds. MiR-30e-3p might influence sorafenib response via CXCL3 regulation modulating stemness and tumour microenvironment infiltration. Increased circulating levels of miR-30e-3p predict the development of sorafenib resistance in a preliminary series of HCC patients and deserve future investigations.

Figure: MiR-30e-3p/p53/MDM2 feedback loops in HCC. Upper panel: miR-30e-3p acts as a tumour suppressor miRNA in TP53 wild type contexts. Lower panels: miR-30e-3p acts as an oncogene in TP53 mutated backgrounds.



PO-195
Usefulness of serum metabolomic profiling for the differential diagnosis in liver cancer: a validation study

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Background and Aims: The early diagnosis of intrahepatic cholangiocarcinoma (iCCA) using noninvasive techniques represents a clinical challenge, as well as the differential diagnosis with hepatocellular carcinoma (HCC). Metabolomics is a powerful source of candidate biomarkers for diagnostic purposes and in a previous study we identified a panel of serum metabolites with better diagnostic capacity than carbohydrate antigen 19-9 (CA 19-9) and alpha-fetoprotein (AFP). The aim of this study was to validate serum metabolomics profiles in patients with iCCA or HCC for the differential diagnosis and to investigate the usefulness in the follow-up.

Method: Metabolites were analysed by ultra-high performance liquid chromatography coupled to mass spectrometry (UHPLC-MS) in serum obtained before any treatment from patients with biopsy-proved diagnosis of iCCA (n=71) or HCC (n=78), and for some of them also in sera obtained one month after curative surgery (iCCA, n=29 and HCC, n=21). Multivariate and univariate analyses of 495 metabolites were carried out.

Results: There was a group of metabolites significantly increased in the serum of patients with liver tumours compared with healthy subjects. The amino acid derivative hypotaurine, the dipeptide phenylalanine-phenylalanine, and the glycerophospholipid PC(O-18:0/18:2) metabolites included in the predictive model generated for discriminating between HCC and iCCA, were also found to be altered in this analysis, which supports the robustness of the biomarkers previously found.
A total of 25 metabolites out of 495 were significantly altered in the comparison between iCCA after and before curative surgery. Of note, serum levels of phosphatidylcholines and acylcarnitines were reduced in iCCA patients after surgery. A total of 46 out the 495 metabolites were found significantly altered in serum of patients with HCC after curative surgery. This profile was characterized by a decrease of 2-aminobutyric and aminoadipic acids and several fatty acids, steroid sulfates and glycerophospholipids. In both cases, some of the metabolites were increased in the serum of patients with liver tumours compared with healthy subjects.

Conclusion: Differential serum metabolomic profiles can be useful for the differential diagnosis of iCCA and HCC and, although the results need to be confirmed, they could help in the follow up after treatment.

PO-199
Liver hypertrophy after parenchyma-sparing major hepatectomy & standard major hepatectomy in the setting of colorectal liver metastases: a comparative multicentre study

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Background and Aims:
Postoperative liver failure (PLF) is linked to the remnant liver volume (RLV), but also related to its regenerative power. Different strategies have been proposed to face this complication. Parenchyma-sparing surgery, aiming to preserve the liver skeleton, is an alternative to standard major hepatectomy (MH). Several reports regarding the regenerative process in MH and staged procedures are proposed. To date, no information in extensive parenchyma-sparing surgery (PSMH) are reported. This study compares the degree of liver hypertrophy (DLH) between patients undergoing PSMH and MH for colorectal liver metastases (CLM).

Method:
The current multi-institutional study was carried out by three HPB centres: Humanitas Research Hospital, Italy; Centre Hepato-Biliaire in Paul Brousse Hospital, France and the Cattinara Hospital of Trieste, Italy. All the consecutive patients undergoing PSMH and MH for CLMs at the 3 centres between 2016 and 2020, were considered. The indication for PSMH was: ≥ 4 bilobar CLMs with at least one lesion in contact with 1st/2nd order portal pedicles or hepatic vein at the caval confluence. The DLH was followed up by contrast-enhanced CT scans performed at 1 week, 1-, 3- and 6 months.

Results:
A total of 71 patients were included: 21 PSMH and 50 MH. In PSMH, the median delta-DLH (compared with RLV) at 1-week, 1-, 3- and 6-months was 33% (range 21-46, p<0.001), 32% (range 17-40, p=0.005), 41% (range 24-48, p=NS), 45% (range 39-49, p=NS), respectively. In MH the median DLH was 17% (range 6-26), 34% (range 14-42), 38% (range 23-49) and 37% (range 24-50). Operative mortality and severe PLF were nil in both groups.

Conclusion:
The present study demonstrates higher early liver hypertrophy after PSMH compared to MH without influencing the PLF rate. These data provide preliminary evidence of the relationship between a surgery skeleton-preserving and the potency of liver hypertrophy. Further analyses have to be done.

PO-202

Direct and indirect antitumoural effects of cold atmospheric plasma: a disruptive technological approach for the local treatment of cholangiocarcinoma

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Background and Aims: Cholangiocarcinoma (CCA) is a rare tumour of the bile ducts characterized by a very poor prognosis (survival rate <5% at 5 years). To date, treatment options are limited and surgical resection, the only effective option, is reserved for resectable patients (<25%). In addition to systemic palliative chemotherapies, it is mandatory to develop new therapeutic options against CCA, in particular local treatments targeting both tumour and its microenvironment. In this line, cold atmospheric plasma (CAP) shows promises in oncology. Generated from the partial ionization of a gas, CAP generates reactive oxygen and nitrogen species that exert deleterious cellular effects leading cell death or dysfunction.

Method: Human cell lines of CCA (EGI-1 and HuCCT1) and of its microenvironment namely cancer-associated fibroblasts (hTERT-HSC, LX2-HSC) and endothelial cells (HUVEC) were treated directly with CAP for 0.5, 1, 3 and 5 minutes. Primary hepatocytes used as non-tumour epithelial cells were isolated from human liver. Cell viability was determined by crystal violet assay. In tumour cells, the induction of immunogenic cell death (ICD) was evaluated *in vitro* by western blot (WT) and by measuring the release of DAMPs in the extracellular environment, such as ATP. Phenotypic and functional changes of cells were analysed by RT-QPCR, WB and video-microscopy.

Results: Our results suggest that CAP can induce antitumour effects that can be direct (death of tumour cells) as well as indirect (dysfunctions of stromal cells, probable activation of immune cells). As a proof of the direct antitumour effects, we have demonstrated that CAP-triggered oxidative stress is responsible for a decreased tumour cell viability while being non-deleterious to healthy epithelial cells, *e.g.* hepatocytes. Interestingly, indirect antitumour effects have also been evidenced considering the effects of CAP on the underlying tumour microenvironment, especially on its cellular components such as macrophages, endothelial cells and fibroblasts. Indeed, CAP decreases the activation state of fibroblasts, and their migration, and inhibits the angiogenic profile of endothelial cells. It turns out that CAP can promote reticulum endoplasmic stress, activate autophagy and drive to the release of DAMPs by malignant cells in the tumour stroma. In turn, these DAMPs could stimulate the surrounding immune cells and promote antitumour immunity.

Conclusion: CAP opens perspectives for local treatment of CCA. In order to transfer this technology to patients, the plasma source has been miniaturized allowing to deliver the plasma *in situ via* an endoscope (patent pending). Feasibility and safety studies of the plasma endoscopic probe in pigs are in progress in close collaboration with clinicians.

PO-203

The probability to be cured after surgery for hepatocellular carcinoma: can these patients reach again the general population life span? A national multicentric epidemiologic study

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Background and Aims: When patients develop cancer, the first think is if they will we be cured definitely or not. In oncology the definition of cure is a complicated concept, particularly in case of hepatocellular carcinoma, that are affected by a high rate of recurrence after a curative intent, and by an underlying liver damage that could modify by itself the prognosis. The aim of this study was to measure in a large multicentric Italian population the probability and the timing of being cured after surgery for HCC in comparison with a standard population.

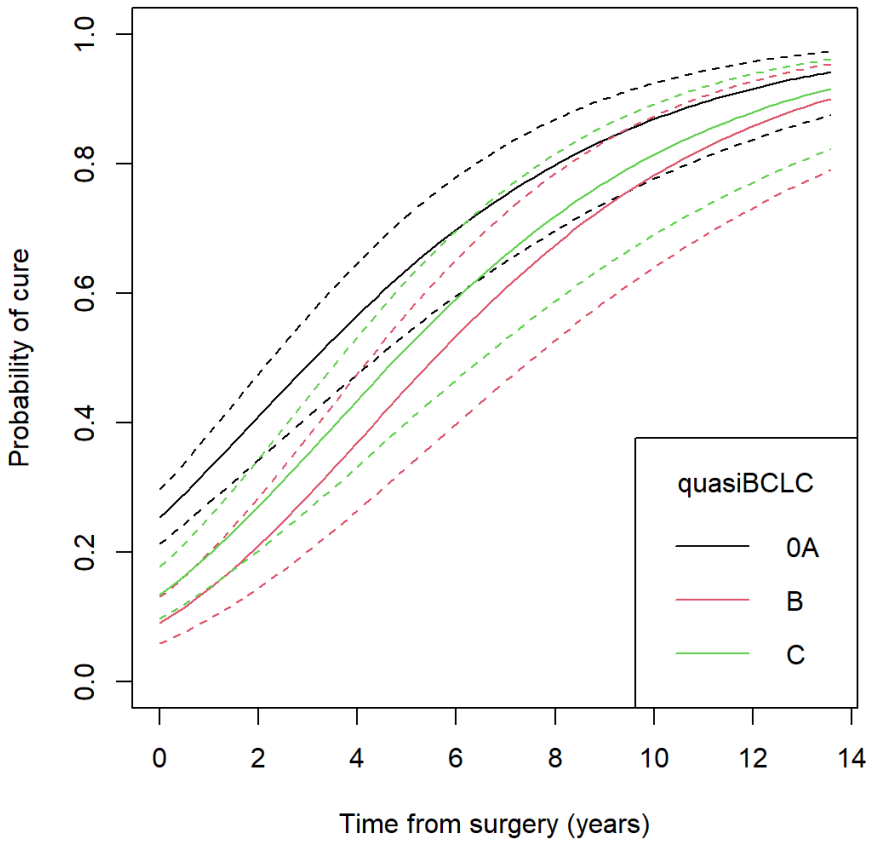
Method: Data were retrieved by the Italian HCC register (HE.RC.O.LE.S.), from 2009 to 2020. A Weibull-non mixture cure model was employed to compare disease-free-survival (DFS) among HCC patients and the general Italian population matched by age, sex and year of diagnosis. The same comparison was then performed subgrouping HCC patients according to their tumour stage.

Results: Among 30 participating centres, 3351 patients were enrolled. The probability of resection to guarantee the cure is 21% (95%CI: 0.17-0.25), and it increase during time reaching 93.8% after 14 years after resection without experiencing a recurrence. Patients who were not cured had a median DFS of 3.2 years (95%CI: 2.7-3.9). According to the tumour stage, BCLC 0-A patients had a cure fraction of 25.5% (95%CI: 0.21-0.29), BCLC B of 9.2% (95%CI: 0.06-1.13) and BCLC C 13.5% (95%CI: 0.09-1.17). In terms of potential years of life lost (PYLL), in case of early recurrence (<2 years after surgery) patients

lost 8.34 years (95%CI: 7.68-8.99) from their life expectancy, and they returned to have almost the same lifespan of the healthy general population after 15 years from surgery.

Conclusion: The chance to be cured after resection for HCC are low, but they increase significantly with the increase of the time without recurrence. However, these patients need at least 15 years before they could be declared cured. These results may help to better inform patients and to ameliorate the follow-up protocols.

Figure:



PO-211
Stratification of cirrhosis etiologies identifies novel immune biomarkers for early stage hepatocellular carcinoma

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Background and Aims: Novel blood biomarkers to predict and detect curable, early stage hepatocellular carcinoma (HCC) is the most effective strategy to improve survival of HCC patients. Others and we have recently published on the applicability of serum immune proteins as early HCC biomarkers, but these studies had limited reproducibility. We now study in detail the contribution of the cirrhotic stage and disease etiology on the circulating immune response, in order to increase the diagnostic robustness of an early HCC immune signature.

Method: A retrospective cohort of 1585 patients with pathology- or radiology-proven HCC was established of patients diagnosed at our large tertiary HCC referral centre, among them were 522 (33%) with BCLC 0/A stage HCC. Immune profiles of a balanced cohort of 188 cirrhotic patients, and 195 early HCC patients with hepatitis B (HBV), hepatitis C (HCV), ALD alcoholic liver disease (ALD) or non-alcoholic liver disease (NAFLD) were determined through serum multiplex profiling.

Results: We show that liver cirrhosis had a profound effect on the levels of circulating immune proteins with significant dysregulation of 24 out of 59 serum immune mediators. Stratification of cirrhosis patients in groups with distinct etiologies identified 45 significant immune mediators and heat map revealed obviously distinct immune profiles in each etiology. HBV-cirrhosis was characterized by high levels of circulating TRAIL and IFN-gamma, HCV-cirrhosis by high levels of IP-10 and the immune profiles of ALD-cirrhosis; NAFLD-cirrhosis were more comparable with higher levels of IL-8, IL-6, CCL25 and LIF. Eight immunological mediators were significant independent predictors of early stage HCC and displayed specificity for one of the four cirrhosis etiologies. These immune mediators improved existing tumours marker efficacy and when combined with AFP detected early stage HCC with an AUC of 0.80.

Conclusion: HCC develops in an inflammatory heterogenetic background. In our search for predictive HCC markers, we found that liver cirrhosis and cirrhosis etiology had a profound impact on the circulating immune response and associated with a wide repertoire of pro-inflammatory and HCC promoting immune mediators. Moreover, we identified Immunological markers that differentiated early stage HCC from cirrhosis. Strict stratification resulted in a set of immune mediators with good sensitivity for early stage HCC of different etiologies

PO-220
Lenvatinib with or without concurrent drug-eluting beads transarterial chemoembolization in patients with advanced hepatocellular carcinoma: A real-world, multicentre, retrospective study

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Background and Aims: Lenvatinib is the first-line treatment for advanced hepatocellular carcinoma (HCC). We aimed to compare the clinical outcomes of lenvatinib plus drug-eluting beads transarterial chemoembolization (DEB-TACE) versus lenvatinib alone in real-world practice.

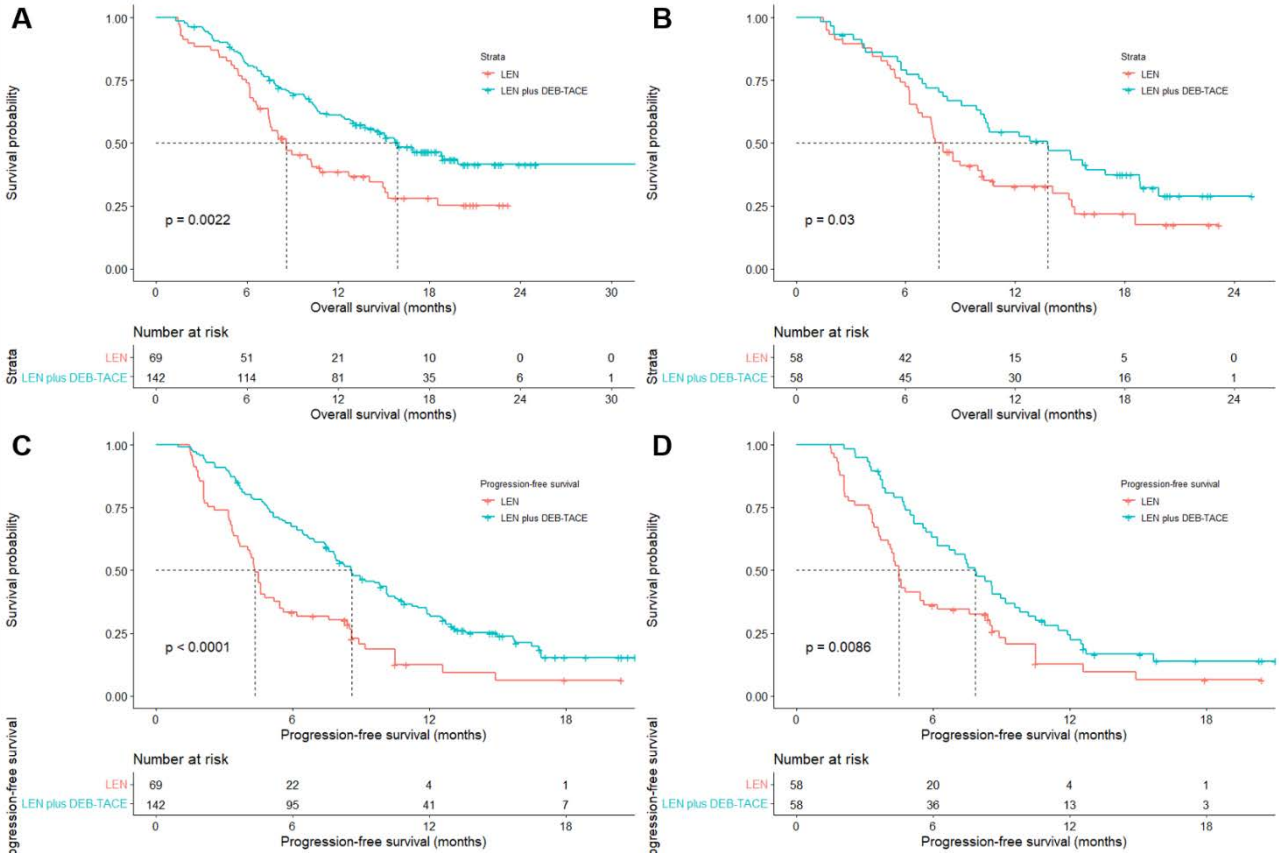
Method: This retrospective analysis included 142 consecutive patients who received lenvatinib plus DEB-TACE and 69 patients who received lenvatinib alone as first-line treatment from 15 Chinese academic centres from Nov 2018 to Nov 2019. Overall survival (OS), progression-free survival (PFS), objective response rate (ORR) evaluated by mRECIST criteria, and safety profiles were compared between the two groups.

Results: The median OS and PFS were significantly longer in the combined therapy group than in the monotherapy group in whole cohort (median OS, 15.9 vs 8.6 months, p=0.0022; median PFS, 8.6 vs 4.4 months, p<0.001) and after propensity score matching analysis (median OS, 13.8 vs 7.8 months, p=0.03; median PFS, 7.8 vs 4.5 months, p=0.009). Moreover, the treatment option was an independent prognostic factor for OS and PFS with adjustment based upon baseline characteristics (adjusted hazard ratio [HR]=0.53, 95% confidence interval [CI]: 0.36–0.78, p=0.001, and adjusted HR=0.42, 95% CI: 0.30–0.60, p<0.001, respectively) and propensity score (adjusted HR=0.52, 95% CI: 0.36–0.76, p=0.001, and adjusted HR=0.46, 95% CI: 0.33-0.64, p<0.001, respectively). Moreover, a greater ORR was observed in the combined group (ORR=46.48% vs. 13.05%, p<0.001). Furthermore, the most common adverse events were elevated AST (54.9%) and fatigue (46.4%) in the lenvatinib plus DEB-

TACE group and lenvatinib group, respectively. Most adverse events were mild-to-moderate and manageable.

Conclusion: With well-tolerated safety, lenvatinib plus DEB-TACE was more effective than lenvatinib monotherapy in improving OS, PFS, and ORR. Thus, it may be a promising treatment for advanced HCC. Future prospective studies confirming these findings are warranted.

Figure: Comparison of OS and PFS between LEN plus DEB-TACE and LEN by Kaplan-Meier method in patients before PSM(A & C, respectively) and after PSM (B & D, respectively)



PO-221
Liver biopsy biomarkers in a phase 1 study of the prodrug MIV-818 demonstrates proof-of-concept for cancer in the liver

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Background and Aims:MIV-818 is an orally administered, liver-directed, nucleotide prodrug that has completed an open-label, multi-centre phase 1 clinical trial in patients with hepatocellular carcinoma (HCC), intra-hepatic cholangiocarcinoma (iCCA) or liver metastases (LM) (NCT03781934). The aim of this exploratory analysis was to assess the pharmacodynamic effects of MIV-818, as monotherapy, on translational biomarkers deoxyribonucleic acid (DNA) damage (phospho-ser129-histone H2AX, pH2AX), proliferation (Ki67), and hypoxia (glucose transporter 1, GLUT1).

Method: Nineteen patients with advanced inoperable HCC (7), iCCA (2), mixed iCCA/HCC (1), and LM from solid tumours (9), were enrolled in the phase 1a and 1b monotherapy part of the study. Needle biopsies containing both tumour and normal liver tissue were collected in cycle 2 of MIV-818 treatment, fixed in 10% neutral buffered formaldehyde and paraffin embedded. Immunohistochemistry (IHC) analysis of pH2AX, Ki67), and GLUT1 was performed on the cycle 2 sample and, if present, an archival/predose sample.

Results: Twelve on-treatment cycle 2 biopsies were taken and analysed by IHC. Positive staining for pH2AX, indicating DNA-damage, was observed in all on-treatment tumour samples, whereas no pH2AX staining (<1% of cells) was observed in healthy liver tissue from the same biopsy. In cases where an archival/predose biopsy was available for comparison, a clear induction of pH2AX was seen after MIV-818 treatment. High levels of pH2AX positive cells were also observed in membrane GLUT1 staining (hypoxic) regions of the tumour. Proliferation was generally low in non-tumour tissue (<10% Ki67 positive cells), with tumour samples varying from 10 to 80% Ki67 positive cells.

Conclusion: Tumour biopsies demonstrated evidence of selective, drug-induced, DNA damage in tumour tissue including hypoxic regions of the tumour. No impact of MIV-818 observed in healthy liver tissue, supporting the proof-of-concept for MIV-818 liver tumour targeting.

PO-224
Notch signalling is significantly altered in KPPTom mice, a bespoke murine model of intrahepatic cholangiocarcinoma

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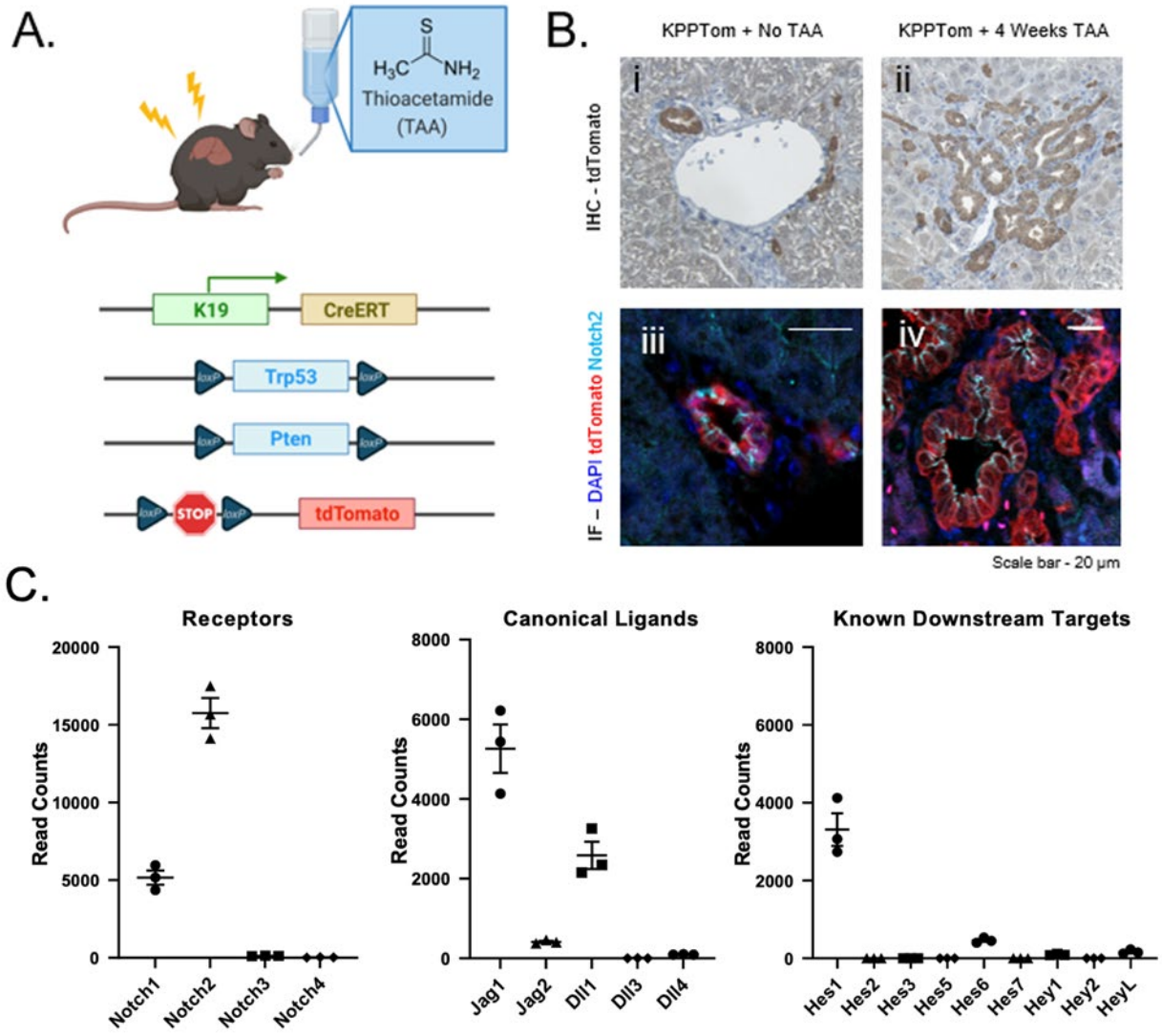
Background and Aims: Notch signalling is a conserved pathway of cell-cell communication that is essential for the formation of bile ducts during development, as well as for biliary repair and regrowth throughout life. Since Notch signalling is a master regulator of biliary growth and repair, we hypothesize that Notch signalling is utilized in the formation of bile duct cancer, intrahepatic cholangiocarcinoma (iCCA), since it arises on the background of hepatic damage and retains many of the morphological features of its ductular origin. Notch signalling is known to be highly elevated in mature iCCA tumours; however, it is not known whether active Notch signalling directly promotes neoplastic transformation of cholangiocytes and early cancer formation. My research aims to define the role of Notch signalling in early iCCA formation.

Method: We have developed a murine model of iCCA called KPPTom, which has inducible deletion of the oncogenic repressors Pten and Tp53 in the biliary epithelium exclusively, as reported by a tdTomato fluorescent reporter. These mice were subjected to treatment with a hepatotoxin, thioacetamide (TAA), for up to 8 weeks. Histological staining and high-resolution confocal imaging were used to determine the relative localization and expression of various Notch signalling components. Bulk RNA sequencing was performed for tdTomato+ cells isolated from KPPTom mice that were treated with TAA for 8 weeks.

Results: KPPTom mice fail to develop biliary tumours unless they are also sustain hepatic injury with TAA, suggesting that both genetic susceptibility to cancer as well as cumulative liver damage are necessary for iCCA formation in vivo. B. Immunohistochemical and immunofluorescent analysis of formalin-fixed liver sections from KPPTom mice treated with TAA for 4 weeks revealed the formation of iCCA tumours (ii and iv), which highly expressed Notch2, one of the four mammalian Notch receptors, apically and at cell junctions between cholangiocytes in cancerous ducts (iv). C. Bulk RNA sequencing revealed significant increases in gene expression of the receptors Notch1 and Notch2, canonical ligands Jag1 and Dll1 and downstream transcriptional target Hes1 in tdTomato+ cholangiocytes.

Conclusion: Our results show that Notch signalling is significantly altered in a bespoke murine model of iCCA, called KPPTom, when these mice are cumulatively treated with the hepatotoxin TAA. Specifically, Notch2 is upregulated in pre-cancerous cells (tdTomato+) and localizes apically and laterally at cell junctions. This work highlights the importance of Notch signalling in early iCCA pathophysiology and identifies potential benefit in targeting specific Notch pathway components in patients with iCCA.

Figure: Notch signalling is significantly altered in KPPTom mice, a bespoke murine model of iCCA



PO-225
BH3 mimetics for precision prevention for beta-catenin mutant hepatocellular carcinoma

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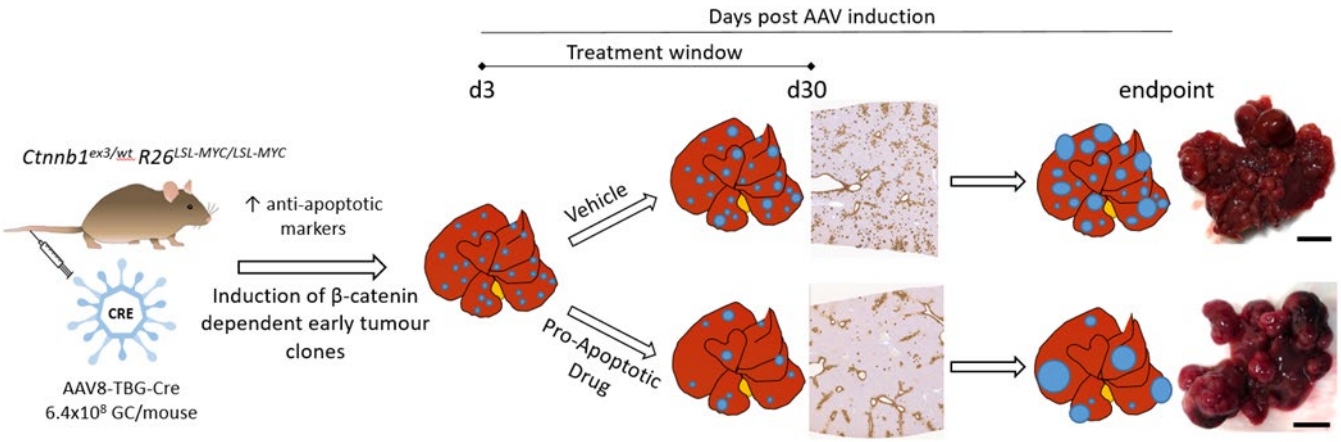
Background and Aims: Most hepatocellular carcinoma (HCC) develops in patients with chronic liver disease, enabling identification of those most at risk of cancer development coupled with screening and attempts towards cancer prevention. Some preventative strategies for HCC are already available, but they are generally marginally effective, untargeted and rely on lifestyle modification typically with related poor adherence. Precision prevention therapy to stop premalignant clones progressing to cancer does not exist currently. Through the development of bespoke genetically engineered mouse models (GEMMs), we have established a platform to study the *in vivo* development of tumours from single initiating clones within the mouse liver. Our aim is to map these early clones over time and space during their expansion into endstage tumours. This allows us to identify vulnerabilities specific to early clones and potential targets for therapeutic HCC prevention.

Method: We use our established model of AAV8-TBG-Cre mediated clonal activation of beta-catenin and overexpression of c-Myc in hepatocytes. This generates malignant lesions similar to human beta-catenin mutant HCC. Guided by transcriptomic analysis performed after early oncogenic transformation we explored the use of BH3 mimetics, which acts as a pro-apoptotic senolytic, as monotherapy to prevent the outgrowth from early tumour clones. *In vivo* prevention trials with the BH3 mimetic ABT263 or the tyrosine kinase inhibitor Lenvatinib, were performed on the background of either a healthy or steatotic liver. Mice were sampled at 1 month after genetic induction and at tumour endpoint and assessed using organotypic assays, immunohistochemistry and *in situ* hybridization.

Results: We identified activation of anti-apoptotic pathways following initial beta-catenin/ c-Myc activation in hepatocytes. In the beta-catenin dependent GEMM, after treatment during the early carcinogenic phase we observed enhanced removal of early oncogenic clones with ABT263 unlike Lenvatinib. This reduction with ABT263 corresponded to reduced clonal tumour number, decreased tumour burden and significantly prolonged survival. When used in late stage disease ABT263 was however, ineffective unlike Lenvatinib which now improved survival

Conclusion: We identify the anti-apoptotic pathway as a means to specifically target tumour clones for cell clearance during early carcinogenesis. Preventative therapy using the BH3 mimetic Navitoclax (ABT263), a relative of the FDA-approved Venetoclax, is well tolerated in mice and identifies this class of therapy as a potential candidate for clinical translation for patients at risk of HCC development.

Figure:


Days post AAV induction
Treatment window
d3
d30
endpoint
Ctnnb1ex3/wt R26LSL-MYC/LSL-MYC
↑ anti-apoptotic markers
CRE
AAV8-TBG-Cre
6.4x10⁸ GC/mouse
Induction of β-catenin dependent early tumour clones
Vehicle
Pro-Apoptotic Drug

PO-231
Early Kupffer cell depletion does not affect hepatocellular carcinoma progression in mice

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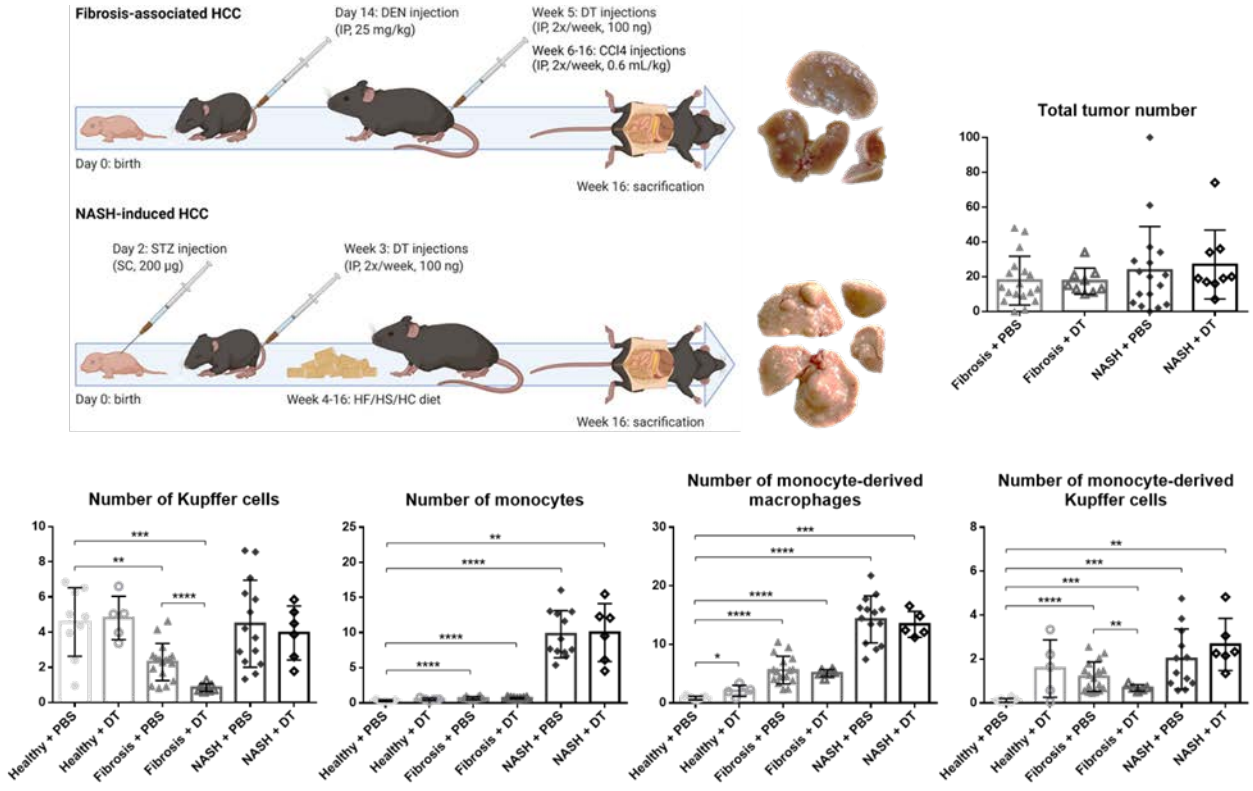
Background and Aims: Hepatocellular carcinoma (HCC) represents the majority of primary liver cancer cases. Its aggressive disease behaviour and poor prognosis substantiate the critical need to urgently address the lack of effective HCC treatment options. HCC usually occurs in a background of chronic liver disease (CLD), characterized by chronic hepatic inflammation and fibrosis, in which Kupffer cells (KCs), resident liver macrophages, have been proposed to play a role. However, the role of KCs in HCC initiation and progression remains unknown.

Method: In order to investigate the role of KCs in HCC initiation, transgenic Clec4F-diphtheria toxin receptor mice were used. Diphteria toxin (DT)-mediated KC depletion was performed prior to the introduction of chronic liver damage in both a fibrosis-associated and a non-alcoholic steatohepatitis (NASH)-induced HCC mouse model.

Results: At the time of sacrifice, macroscopic hepatic tumours were present in approximately 100% of the cases in both HCC mouse models without differences between groups. Flow cytometric analysis of the liver tissue showed depletion of KCs and infiltration of monocytes and monocyte-derived macrophages (MoMfs), confirming previous literature, and the presence of monocyte-derived KCs (MoKCs) in both models. DT-mediated KC ablation at the initiation stage of HCC induction resulted in a decreased number of KCs and MoKCs at end-stage disease in fibrosis-associated HCC but not in the model of NASH-induced HCC. This altered hepatic macrophage pool composition was however not associated with significant changes in tumour burden, HCC markers or inflammatory, angiogenic and fibrotic components of the tumour microenvironment (TME). In NASH-induced HCC, depletion of the KCs resulted in decreased hepatic expression of alpha-smooth muscle actin and vascular endothelial growth factor, however, no differences were detected on histology for tumour burden, steatosis, inflammation and fibrosis, substantiating the limited effect of KC depletion in the initiation phase of HCC pathogenesis.

Conclusion: Despite the tolerogenic function of KCs in homeostasis and the reported role as early activators during inflammation, depletion of KCs during the initiation phase of HCC pathogenesis only has minor effects on the TME and does not affect disease severity or progression in HCC mouse models with different underlying backgrounds.

Figure:


Fibrosis-associated HCC
Day 14: DEN injection
(IP, 25 mg/kg)
Week 5: DT injections
(IP, 2x/week, 100 ng)
Week 6-16: CCl4 injections
(IP, 2x/week, 0.6 mL/kg)
Day 0: birth
Week 16: sacrification
NASH-induced HCC
Day 2: STZ injection
(SC, 200 µg)
Week 3: DT injections
(IP, 2x/week, 100 ng)
Day 0: birth
Week 4-16: HF/HS/HC diet
Week 16: sacrification
Total tumor number
Fibrosis + PBS
Fibrosis + DT
NASH + PBS
NASH + DT
Number of Kupffer cells
Number of monocytes
Number of monocyte-derived macrophages
Number of monocyte-derived Kupffer cells
Healthy + PBS
Healthy + DT
Fibrosis + PBS
Fibrosis + DT
NASH + PBS
NASH + DT

PO-232
NOL9 Promotes the Proliferation and Suppresses the Apoptosis of Hepatocellular Carcinoma Cells by Suppressing the JAK2/STAT3 Pathway

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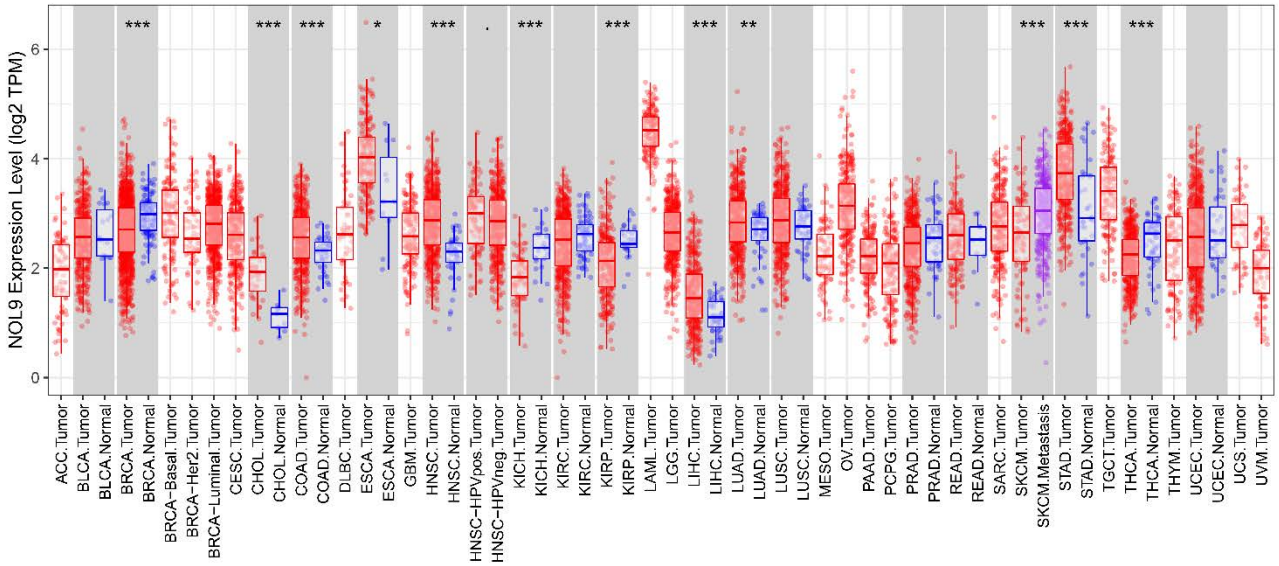
Background and Aims: Liver cancer is the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death. However, the pathogenesis of HCC is still unclear currently, so it is difficult to find a definitive and effective treatment. In this study, we investigate the role and function of NOL9, a protein related to ribosome biogenesis, in hepatocellular carcinoma (HCC).

Method: The expression of NOL9 was analysed in The Cancer Genome Atlas (TCGA) and genotype-tissue expression pan-cancer data. The association between NOL9 expression and patient prognosis was evaluated using TCGA clinical survival data. Enrichment analysis of NOL9 was performed using the clusterProfiler R software package. Moreover, the correlation between NOL9 expression and immune cell infiltration were evaluated by analysing Tumour IMmune Estimation Resource (TIMER) web server. Then, lentivirus-mediated loss-of-function experiments were used to explore the function of NOL9 in cell proliferation ability and cell apoptosis of HCC lines HCCLM3 and PLC/PRF/5. Clinical samples were used to confirm that NOL9 expression is increased in tumour tissue and is associated with poor prognosis of HCC patients. Western blot and xenograft were performed to evaluate the role of NOL9 on signaling pathways in HCC.

Results: NOL9 was highly expressed in many types of tumours including HCC. High NOL9 expression was significantly correlated with immune inflammation-related gene sets. Further analysis showed that high NOL9 expression was associated with high macrophage, neutrophil and dendritic cell infiltration scores. Moreover, NOL9 knockdown inhibited the cell proliferation ability and promoted cell apoptosis in HCCLM3 and PLC/PRF/5. Further, high NOL9 expression correlated with poor prognosis of HCC patients. Mechanistically, Western blot results have demonstrated that low NOL9 expression suppressed the activation of JAK2/STAT3 signaling pathway.

Conclusion: Our study reveals that low NOL9 expression inhibits the JAK2/STAT3 signaling pathways, thus inducing the apoptosis and suppressing the cell proliferation.

Figure:


NOL9 Expression Level (log2 TPM)
0
2
4
6
***
***
***
*
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**
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***
***
ACC.Tumor
BLCA.Tumor
BLCA.Normal
BRCA.Tumor
BRCA.Normal
BRCA−Basal.Tumor
BRCA−Her2.Tumor
BRCA−Luminal.Tumor
CESC.Tumor
CHOL.Tumor
CHOL.Normal
COAD.Tumor
COAD.Normal
DLBC.Tumor
ESCA.Tumor
ESCA.Normal
GBM.Tumor
HNSC.Tumor
HNSC.Normal
HNSC−HPVpos.Tumor
HNSC−HPVneg.Tumor
KICH.Tumor
KICH.Normal
KIRC.Tumor
KIRC.Normal
KIRP.Tumor
KIRP.Normal
LAML.Tumor
LGG.Tumor
LIHC.Tumor
LIHC.Normal
LUAD.Tumor
LUAD.Normal
LUSC.Tumor
LUSC.Normal
MESO.Tumor
OV.Tumor
PAAD.Tumor
PCPG.Tumor
PRAD.Tumor
PRAD.Normal
READ.Tumor
READ.Normal
SARC.Tumor
SKCM.Tumor
SKCM.Metastasis
STAD.Tumor
STAD.Normal
TGCT.Tumor
THCA.Tumor
THCA.Normal
THYM.Tumor
UCEC.Tumor
UCEC.Normal
UCS.Tumor
UVM.Tumor

PO-235
Disease positioning identifies distinct hepatocellular carcinoma subtypes within genetically engineered mouse models which transcriptionally relate to equivalent subclasses in human hepatocellular carcinoma

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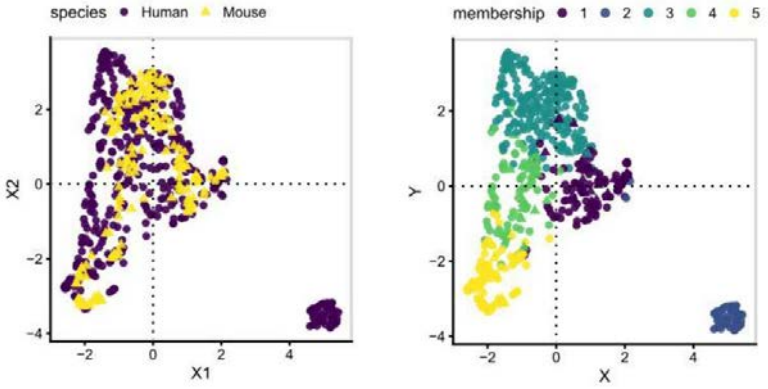
Background and Aims: The systemic treatment landscape for advanced hepatocellular carcinoma (HCC) has expanded significantly over the last decade, with multiple drugs/modalities now approved for clinical use. This propels HCC therapy forward into a space where treatment options could be tailored to the patients in a precision medicine approach, rather than a one-size-fits-all. However, to guide informed decisions and enable development of subclass-specific treatments well-characterised pre-clinical models representing the different human HCC subclasses are needed. Our aim was to develop a suite of genetically engineered mouse models (GEMMs) and link them to human subtypes of HCC to ultimately identify and test new therapies with human-relevant pre-clinical models.

Method: We transcriptomically characterized over 20 newly developed GEMMs, in addition to a range of orthotopic cell line transplant and classical toxicological mouse models. These were then disease positioned against a TCGA dataset of human HCC. We used a Louvain clustering algorithm to identify distinct groups within the human/mouse dataset. We then performed pathway enrichment analysis using GSEA Hallmark sets. Furthermore, we validated the subclasses histologically comparing human and mouse tissue and performed organoid-sensitivity guided therapeutic studies in vivo.

Results: When compared to several human cancer types our mouse models most closely correlated to human HCC. However, we were not able to satisfactorily cluster the mice and the human HCC samples based on the transcriptomic data utilizing previously defined human subclasses, such as Hoshida (S1-S3) or Boyault (G1-G6). Thus, we employed a Louvain clustering algorithm and identified 5 distinct subclasses within the human data samples with 4 overlapping mouse subclasses. Each subclass has distinct features, such as well-differentiated, highly proliferative, or strong inflammatory signals. These features also translate into histopathological phenotypes when cross-comparing mouse and human. Importantly, treatment responses in the avatar mice differ between subtypes.

Conclusion: We have identified specific mouse models representing distinct subclasses of human HCC. These models provide a platform for both detailed interrogation of the biological mechanisms driving HCC and for informing translational research. This subtype-specific approach may guide future precision medicine development for HCC patients.

Figure:



PO-241
ZEB1 expression mediates chemoresistance in cholangiocarcinoma by disrupting the pro/anti-apoptotic balance in stromal myofibroblasts

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Background and Aims: Cholangiocarcinoma (CCA) is characterized by a prominent fibrous stroma mainly composed of cancer-associated fibroblasts (CAF) and a poor prognosis due to its late clinical presentation and the lack of effective non-surgical treatments. ZEB1 is a transcription factor expressed by tumour and stromal cells that contributes to the acquisition of metastatic, stem cell properties and chemoresistance. We recently reported a role for ZEB1 in the activation and secretory phenotype of CAF from CCA. Here we aimed to unravel the role of ZEB1 in the chemoresistance mechanisms of stromal CAF.

Method: Immunohistochemical analyses were performed to determine ZEB1 expression in CAF from human CCA. Loss-of-function cellular models were generated by lentiviral infection in hepatic stellate cell (HSC) derived cell lines (hTERT-HSC and LX2-HSC) as models of CAF. Cell viability was determined by crystal violet assay. The expression of ZEB1 and markers of chemoresistance mechanisms were evaluated *in vitro* by RT-QPCR, Western blot and immunofluorescence. *In vivo* experiments were performed using a xenograft CCA model.

Results: ZEB1/alpha-smooth muscle actin co-immunostaining showed ZEB1 expression in CAF from human CCA in 100% of the 45 cases evaluated. Analysis of mRNA and protein expression revealed high ZEB1 and alpha-SMA expression in hTERT-HSC, close to that in CAF isolated from CCA, but lower expression in LX2-HSC. ZEB1 downregulation in hTERT-HSC and LX2-HSC rendered the cells more sensitive to gemcitabine and cisplatin, the current chemotherapeutic drugs used in CCA, but not to sorafenib, current standard of care in hepatocellular carcinoma. The increased sensitivity to gemcitabine and cisplatin was not accompanied by changes in the mRNA expression of known uptake or export transporters for these drugs. However, ZEB1-downregulated cells showed downregulation in the expression of anti-apoptotic gene *BCL2* and upregulation of pro-apoptotic genes *PMAIP1*, *BCL2L11* and *BID*. Thus, exposition to gemcitabine and cisplatin was more efficient inducing apoptosis in ZEB1-downregulated cells, as ascertained by a higher increase in the expression of gammaH2AX and p21, a higher phosphorylation of CHK1 and p53, and a more intense staining of cleaved PARP, compared to the control cells. *In vivo*, tumours developed from human CCA cells treated with gemcitabine showed an increase in the expression of pro-apoptotic genes in the murine stromal compartment. Furthermore, ZEB1 expression correlated inversely with that of the pro-apoptotic gene *BAX* in CCA samples from the TCGA cohort.

Conclusion: ZEB1 expression mediates chemoresistance in CAF from CCA by disrupting the pro/anti-apoptotic balance.

PO-248
Machine-learning predictive model for the optimal treatment allocation in recurrent hepatocellular carcinoma after surgery. A multicentric study with international validation.

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Background and aims: Recurrent HCC (HCCr) has still not a clear indication about the choice of the best potential treatment (BPT) for patients who experiment recurrence after surgery. There are several treatments for relapse: curative treatments (CUR, as redo-surgery or thermoablation), Sorafenib (SOR) and chemoembolization (TACE). The aim of this study is to allocate the patient to their BPT creating a SAR-based (Survival After Recurrence) machine-learning predictive model.

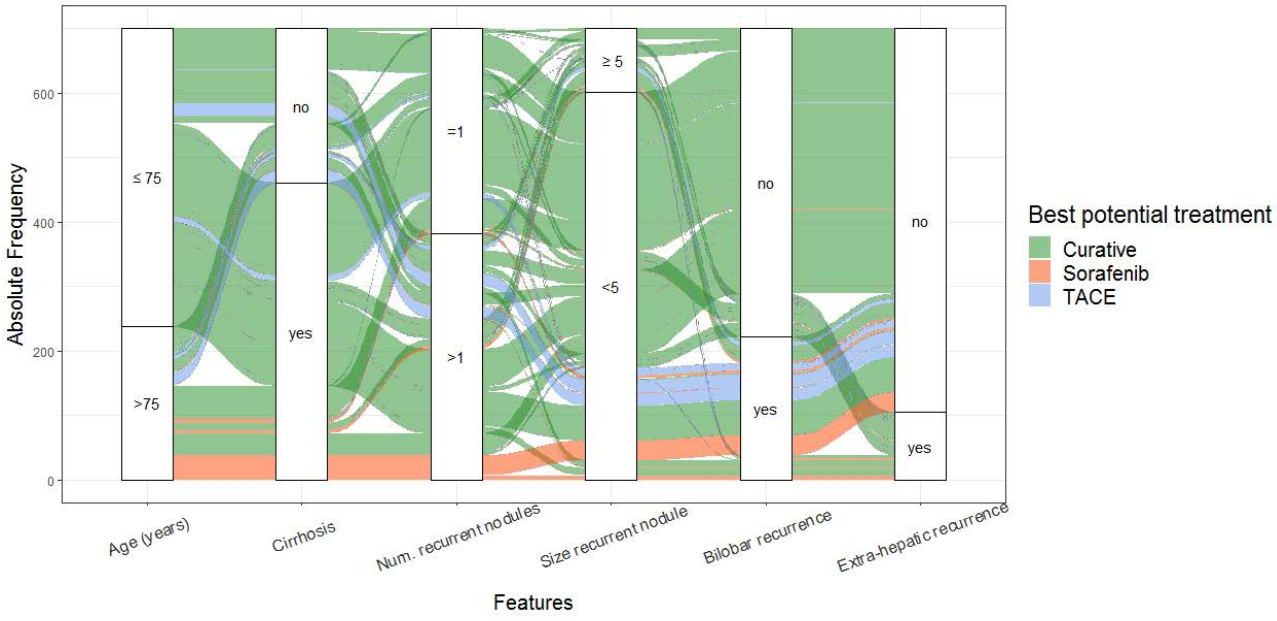
Method: Patients with HCCr were achieved from HE.RC.O.LE.S. registry (Italian registry of hepatocellular carcinoma). Factors predicting SAR were assessed and combined with all the possible treatments, these combinations play differents role as treatment-effect modifiers (TEM). TEM and SAR predictors were: treatment, age, cirrhosis, number, size and lobar localization of the recurrent nodules,

extra-hepatic spread and time to recurrence. The model was assembled into tree-diagram and BPT web-app has been created and then released. Data derived from the ITA.LI.CA. Italian Registry and the University of Tokyo (Japan) were used for the external validation.

Results: 701 HCCr patients (enrolled between 2008 and 2019) met the inclusion criteria. Relapse treatments are divided as follows: 293 (41.8%) curative treatments, 188 (26.8%) sorafenib, and 220 (31.4%) TACE. 5-year after recurrence AUC for overall survival (OS) was 79.4% for BPT web-app predictive model compared with 76.1% in the ITA.LI.CA. register(n=295) and 70.9% in the Japanese cohort (n=422).
Considering potential SAR, the model predicts a benefit for 611 patients (87.2%) referred for curative treatment, 37 (5.2%) for sorafenib and 53 (7.6%) for TACE.
Patients assigned to best potential treatment with sorafenib (BPT-SOR) or TACE (BPT-TACE), are statistically significantly older (p <0.001), than BPT-CUR, and also had a lower median number of multiple recurrent nodules (p = 0.027). Extrahepatic recurrence was observed in 43.2% of BPT-SOR, vs. 14.6% for BPT-CUR, and 0.0% for BPT-TACE (p<0.001). All these findings were employed to create profiles on which a patient-specific algorithm for the BPT allocation was shaped.

Conclusion: The best choice for patients with relapse is a curative approach, but it has been proposed in less than a third of cases. The algorithm proposed in this study could help select the BPT for HCCr patients based on the characteristics of each patient in a treatment hierarchy fashion.

Figure:



PO-259
Response to sorafenib in HCC is mediated by hypoxia-related proteins and induces distinct immune-related signatures

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Background and aims: Sorafenib was the only approved systemic therapy with demonstrated survival benefit in advanced stage of hepatocellular carcinoma (HCC) for more than a decade. Despite the current success of the multi-tyrosine-kinase inhibitor sorafenib, many of the patients respond poorly to the drug treatment or relapse quickly after initial remission. Therefore, our goal was to dissect molecular drivers of drug resistance to sorafenib and to identify novel prognostic markers associated with distinct tumour immune landscapes.

Method: From a cohort of 91 patients treated with sorafenib, we identified 19 HCC patients with particularly good and bad response to the treatment. Integrative RNA sequencing and whole-exome sequencing analyses were performed to identify predictive markers of sorafenib resistance and associated molecular alterations. *In vitro* validation of defined targets was 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=12). Molecular analyses revealed that poor response to sorafenib was associated with activation of ERK, Ca^{2+} signaling, Hippo/YAP signaling, as well as hypoxia-related scaffold proteins from the 14-3-3 protein family, typically associated with drug resistance and adverse properties. Furthermore, a shift in immune-cell composition with predominant enrichment of M2-immunosuppressive macrophages in worst responders was observed. From hypoxia-related targets modulation of Ca^{2+} signaling with subsequent upregulation of 14-3-3 zeta and sigma proteins was associated with the acquisition of drug resistance. Inhibition of these proteins by specific inhibitors or siRNA in sorafenib-resistant hepatoma cells showed significant reduction in cell proliferation and viability.

Conclusion: Defining the actionable targets of resistance and their subsequent inhibition, i.e., 14-3-3 zeta and/or sigma protein might be an important step to delineate distinct molecular alterations driving sorafenib resistance. Further, changes to the composition of the immune micromilieu in different subgroups could be of particular importance to delineate treatment resistance and warrants further investigations.

PO-264
Benefits of tailored HCC surveillance programs on case-fatality rate and cancer-specific mortality using a modelling approach

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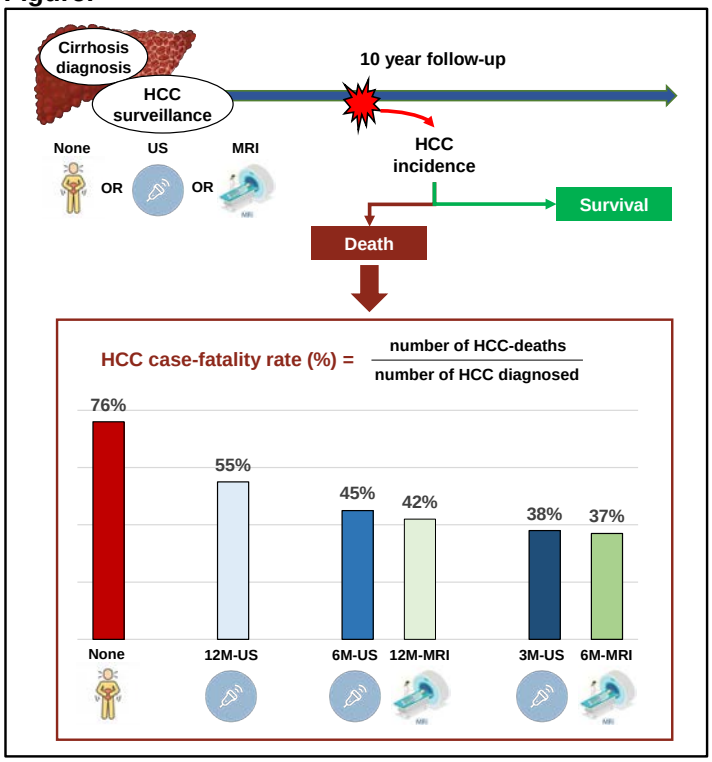
Background and aims: To validate screening programs of solid tumours, expert guidelines recommend obtaining data supporting reduction of case-fatality rate and cancer-specific mortality. This study evaluates the benefit of hepatocellular carcinoma (HCC) screening on those outcomes using a modelling approach.

Method: We designed a Markov model to assess 10-year outcomes of HCC surveillance in terms of case-fatality rate (CFR), disease-specific mortality and overall mortality per 100,000 screened patients with compensated cirrhosis. The model simulates the occurrence of HCC, diagnosis through surveillance or symptoms according to tumour growth, treatment, and follow-up. We evaluated screening in varying scenarios of annual HCC incidence (0.2%, 0.4% or 1.5%), surveillance interval (none, annual (12M), semestrial (6M) or trimestrial (3M)) and imaging modality (US, ultrasound, or MRI, magnetic resonance imaging).

Results: 6M-US screening in comparison to no surveillance reduced 10-year HCC-CFR from 76 to 45%. According to the size of the main tumour, CFRs decreased from 42 to 36% for main nodule size between 1-3cm, 68 to 61% for main nodule size between 3-5 cm, and 83% to 82% for main nodule size larger than 5 cm. When annual incidences varied from 0.2%, 0.4% to 1.5%, the model predicts reductions of 289, 580 to 2,110 HCC-related deaths, and of 209, 419 to 1,521 total deaths, per 100,000 screened patients, respectively. In terms of surveillance modalities, 6M-MRI and 3M-US, in comparison to the recommended 6M-US, yielded reductions of CFRs and cancer-related mortality (-18% and -16%), that were as expected not affected by the variation of incidence. Conversely, effects on overall mortality varied according to annual HCC incidence. In comparison to 6M-US, for incidences varying from 0.2%, 0.4% to 1.5%, 6M-MRI reduced overall mortality from 0.3%, 0.5% to 1.7%, and 3M-US from 0.2%, 0.5% to 1.5%, respectively. Even with extended surveillance interval, 12M-MRI compared to 6M-US leads to a 6% reduction of cancer-related mortality.

Conclusion: Shortening US surveillance or using MRI would reduce HCC case-fatality rate and HCC-related mortality. Conversely, the effect on overall mortality appeared modest. The evaluation of case-fatality rates, absolute numbers of cancer-related and overall deaths, provides additional insights on HCC surveillance.

Figure:


Cirrhosis diagnosis
HCC surveillance
10 year follow-up
None
US
MRI
OR
OR
HCC incidence
Survival
Death
HCC case-fatality rate (%) = number of HCC-deaths / number of HCC diagnosed
76%
55%
45%
42%
38%
37%
None
12M-US
6M-US
12M-MRI
3M-US
6M-MRI

PO-266
Proteomic profiling for theranostic approches in hepatocellular carcinoma

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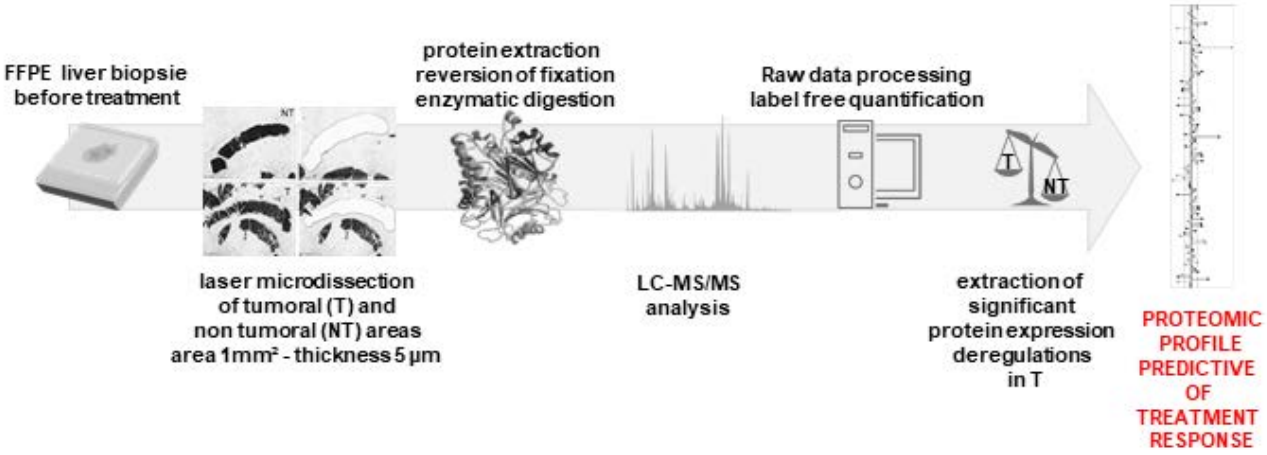
Background and aims: Multiplication of treatments in oncology lead to a new dilemma for oncologists who have to choose the most efficient. Today, there is no efficient tool to guide this choice, as for hepatocellular carcinoma (HCC). HCC is the most common liver cancer and the second leading cause of cancer death worldwide. Due to late diagnosis, its prognosis is extremely poor and most of advanced HCC are only eligible for systemic palliative therapies. After exclusive monotherapy with sorafenib, introduction of new tyrosine kinase inhibitors and immunotherapies is a revolution. However, no rational choice of treatments is defined.
Previously, we demonstrated that biopsies proteomic profiling has diagnostic and prognostic values. Here, we investigated whether this proteomic profile could predict treatments response in HCC.

Method: We analysed HCC proteomic profiles from fixed biopsies before sorafenib treatment. We explored protein expression deregulations between HCC tumours and their adjacent liver tissues. Comparing the proteomic profiles of objective response and progression to sorafenib, we extracted a predictive response signature of 117 proteins. We compared the proteomic profile of patients with an effective response or progression on lenvatinib to this sorafenib response signature.

Results: Using this in situ proteomic strategy, we have demonstrated that HCCs with different responses to sorafenib or to lenvatinib expressed significantly different proteomic profiles. Some proteins of these profiles could be involved in modulating treatment sensitivity. To support this hypothesis, we selected the transketolase (TKT) which was among the most robust and recurring intra-group protein of the predictive response profile for sorafenib. Modulation of TKT expression affected the response to sorafenib in vitro but not to lenvatinib.

Conclusion: This study paves the way for theranostic strategies using proteomic profiling for patient/treatment matching in order to potentiate efficiency of all therapeutic solutions.

Figure: Workflow analysis for tissular proteomic profiling.



PO-267
Comparative in vitro evaluation of cancer therapies in various hepatocellular carcinoma experimental models

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Background and aims: Hepatocellular carcinoma (HCC) is a heterogeneous malignancy. Treatment for advanced HCC has modest effects on improving patient survival, partly attributed to cellular heterogeneity. Several studies categorized HCC into subtypes based on unique cell types and shared molecular profiling. This study aims to evaluate HCC cellular heterogeneity and discover target proteins for potential therapeutic efficacy.

Method: Molecular profiling signatures from different HCC subtypes were retrieved from databases. *In silico* analysis using gradual filtering by protein-protein interaction (PPI) network analysis (Cytoscape) of these signatures identified 20 proto-oncogenes as common potential pharmacological targets. To assess these targets, we used 6 *in vitro* models of epithelial mesenchymal transition (EMT)-transformed phenotype HCC cell lines: JHH6, HLE, and HLF (aggressive), HepG2 and Huh7 (less aggressive) and an immortalised hepatocytes (IHH). Cellular immunophenotype was profiled using HCC stemness markers by flow cytometry. Three cancer therapies (5uM 5-Azacytidine (5-AZA), 50 uM Sorafenib (SOR), and 20 nM PD-L1 silencing (siR-PDL1)) were evaluated. Gene expression analysis was performed to investigate the above common targets' dysregulations upon treatments.

Results: Flow cytometer analysis using HCC stemness markers confirmed the heterogeneity of HCC experimental models. Following treatments, mRNA analysis showed differential results across experimental models, in particular the down-regulation of mRNA expression of FGR/Src and FOS/AP-1 (Table 1). Among 6 cell lines, FGR was downregulated in 4 for 5-AZA, 3 for SOR, and 6 for siR-PDL1, while FOS was down-regulated in 5 for SOR and 6 for siR-PDL1. FGR was decreased upon 5-AZA in HepG2 and IHH for about 100-fold and 25-fold, respectively, and around 2-fold for both HLE and HLF. Notably, sir-PDL1 treated cells showed FGR downregulation in all cell populations (2-, 7-, and 9-fold for HLE, HLF and JHH6 respectively; 5- and 3-fold for HepG2 and Huh7, and 4-fold for IHH). FOS was down-regulated upon SOR for around 6-fold for both HepG2 and Huh7, while it was 5- and 3-fold for HLE and HLF. For siR-PDL1 knockdown cells FOS was downregulated in all cells. Based on treatment types, SOR was effective for most of the proto-oncogene targets in less aggressive EMT-transformed cells vs aggressive EMT-transformed cells, while siR-PDL1 was efficient for all HCC subtypes.

Conclusion: This study indicates the relevance of cellular heterogeneity in response to cancer therapies. Our data identify proto-oncogenes such as FGR/Src and FOS/AP-1 that can be further modulated to improve the efficacy of HCC therapy. Immune targeted therapy such as seems to be a good approach to overcome cellular heterogeneity.

Figure: Table 1. FGR and FOS dysregulation among cell lines after various treatments.

proto-oncogene target	Hepatocytes	aggressive EMT-transformed subtype			less aggressive EMT-transformed subtype		*Cancer therapy*
	IHH	HLE	HLF	JHH6	HepG2	Huh7	
FGR	⇩0.04	⇩**0.48** **	⇩**0.37** *	⇧2.84	⇩**0.01** *	⇧1.51	*5-AZA*
	⇩**0.31**	⇧1.25	⇩**0.06** *	⇧**4.91** ***	⇩0.93	⇧1.44	*SOR*
	⇩**0.24** ***	⇩**0.49** *	⇩**0.14** ***	⇩**0.11** ***	⇩**0.21** ***	⇩**0.36** *	*sir-PDL1*
FOS	⇧4.18	⇩**0.20** *	⇩**0.29** *	⇩0.86	⇩**0.16** **	⇩**0.17**	*SOR*
	⇩**0.38** ***	⇩**0.73** ***	⇩**0.39** ***	⇩0.34	⇩0.80	⇩0.82	*sir-PDL1*

*p≤0.05; **p≤0.01; ***p≤0.001

PO-272
Senescence-specific adaptive immune response to oncogene-induced-senescence (OIS) within the murine liver

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Background and aims: Clinical studies have shown that chronic liver disease is associated with the accumulation of senescent cells within the liver. Senescence is acutely tumour-suppressive, but chronically pro-tumourigenic. Senescent cells signal to immunocytes through a complex secretome, to trigger their own CD4+ T-cell dependent destruction, termed senescence surveillance. The form and functionality of this adaptive immune reaction and why it fails remains unclear.

Method: The Antigen-Receptor Signalling Reporter (AgRSR) mouse permits lineage tracing of T-Cell Receptor (TCR)-stimulated lymphocytes through expression of Cre-recombinase and mKate2, under the control of the *Nur77* (an immediate early gene) promoter. We used hydrodynamic tail vein (HDTV) delivery of $NRAS^{G12V}$-containing transposons, inducing hepatocyte oncogene-induced senescence (OIS) in the AgRSR mouse, before analyses of the immune reaction with flow cytometry.

Results: OIS hepatocytes are cleared between 6 and 12 days after HDTV induction in the AgRSR mouse. At D9 after HDTVi, flow cytometric analysis detected 3.0% ± 1.9 (n=7) of fluorescently labelled intrahepatic CD4+ T-helper during senescence (*Fig.1i & ii*). All were CD44+, indicative of an effector phenotype. Functional *ex vivo* studies showed that 28.9% ±3.6 (n=6) of intrahepatic CD4+ lymphocytes were found to secrete IFNy, characteristic of Th1 polarisation, similar to the control condition ($p>0.05$) (*Fig1. iii*). However, 5.2% ±1.3 (n=8) of intrahepatic CD4+ T-cells were positive for RORyt, the master regulator of Th17 T-helper subsets, compared to 2.6% ±0.8 (n=9) in the non-oncogenic $NRAS^{G12V/D38A}$ control ($p=0.0002$) (*Fig1. iv*).

Conclusion: We have identified senescence-specific CD4+ T-cells during senescence surveillance. Functional analyses of intrahepatic CD4+ lymphocytes suggest an accumulation of functional Th17 cells during active senescence surveillance. Further analysis of the functional properties of senescence-specific CD4+ T-lymphocytes is on-going.

Figure:

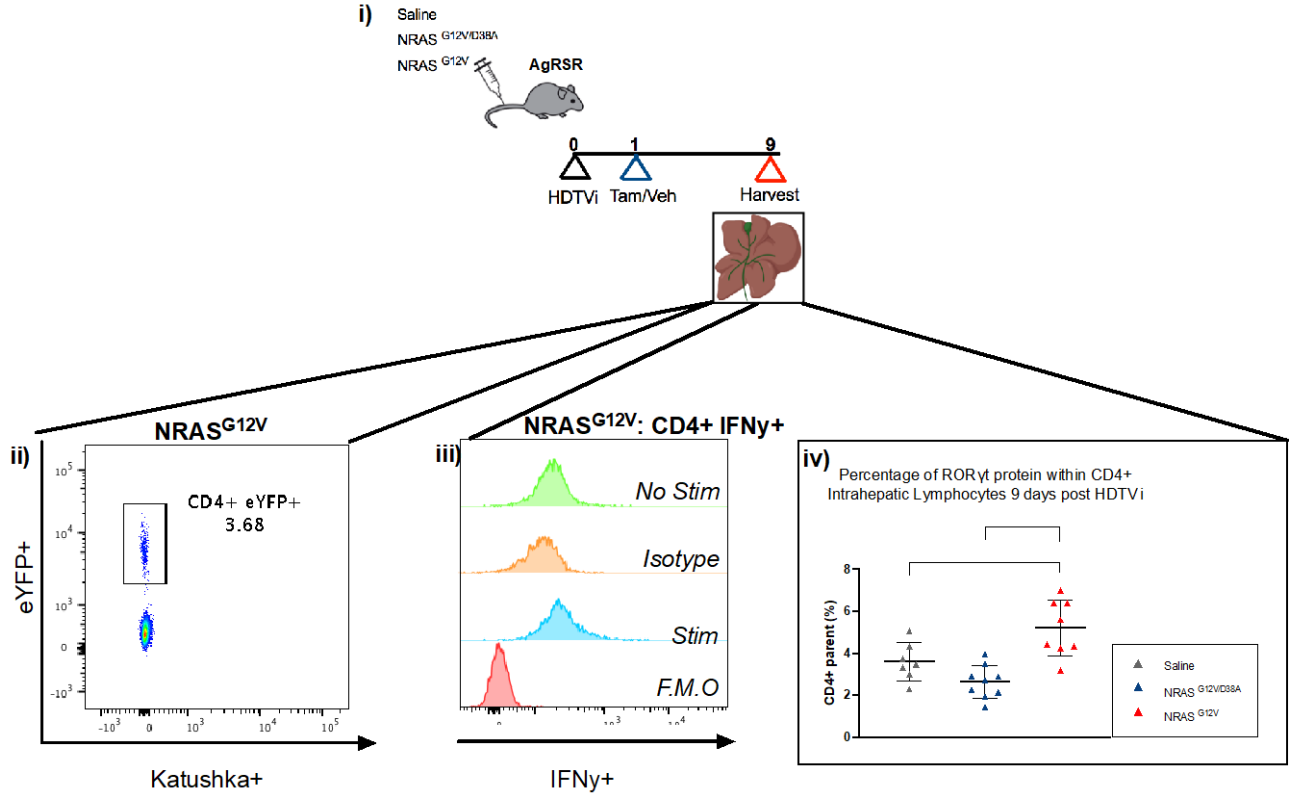

Figure 1: Schematic of experimental workflow and summary of results.

PO-273
Role of EZH2 in the epigenomic landscape of HBV-related liver carcinogenesis

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Background and aims: Hepatocellular carcinoma (HCC) are the fourth leading, fast rising, cause of cancer death worldwide. HCC development is driven by viruses (HBV/HCV) and chronic metabolic alterations leading to chronic inflammation, DNA damage, epigenetic and genetic changes. Epigenetic changes, that include DNA methylation and histone modifications, occur early in the development of HCC and contribute to HCC progression. The histone methyltransferase enhancer of zeste homolog 2 (EZH2) is frequently overexpressed in HCCs. As part of PRC2 complex catalyses the trimethylation of H3K27 (H3K27me3) to repress genes that regulate development and cell differentiation and tumour suppressor genes (canonical activity). Interestingly, in several cancers it has been showed that Ezh2 can also activate transcription, independently of PRC2 (non-canonical function). The aim of our project is to uncover the canonic and non-canonic role of Ezh2 in HBV-infected HCCs.

Method: High-throughput sequencing of anti-EZH2, H3K27me3 and H3K27Ac chromatin immunoprecipitation experiments (ChIP-Seq) in non-infected and HBV-infected HepG2-NTCP cells.

Results: We performed an EZH2 ChIP-seq to identify the complete repertoire of PRC2 targets in non infected and HBV-infected HepG2-NTCP cells. We found 8405 genomic loci with an EZH2 peak in HepG2-NTCP cells. Of note, 2331 out of 8405 are detected only in HBV-infected cells, suggesting a role of HBV in the modulation of EZH2 recruitment. Next, to define which EZH2 target genes are transcriptionally activated or repressed, we performed H3K27me3 and H3K27Ac ChIP-seq in HepG2-NTCP cells. The percentage of the recruitment of EZH2 and the enrichment in acetylation and methylation on lysine 27 of H3 is shown in figure 1. Cross-analysis of EZH2, H3K27me3, H3K27Ac ChIP-Seq and TCGA RNAseq data sets allowed us to define the repertoire of EZH2 target genes deregulated following HBV infection and possibly relevant in cancer and to classify them into canonical (repressed) and noncanonical (activated) EZH2 targets. This analysis allowed to identify 823 canonical PRC2 targets and 725 non-canonical EZH2 targets, confirming the bivalent role of EZH2 in HCC (figure 2). EZH2 repressed genes in non-infected cells enrich several pathways associated with apoptosis, whereas the EZH2 noncanonical transcriptionally activated targets are involved in metabolic pathways or NFKB signaling. 100 EZH2 non-canonical transcriptionally activated targets genes are upregulated in HBV-related patients with HCC and enrich pathways associated with cell cycle.

Conclusion: Altogether, our results define the global repertoire of canonical and non-canonical EZH2 target genes in HBV-infected cells and provide key knowledge for a rational use of anti-EZH2 targeting drugs in anti-HCC combination therapies.

Figures:

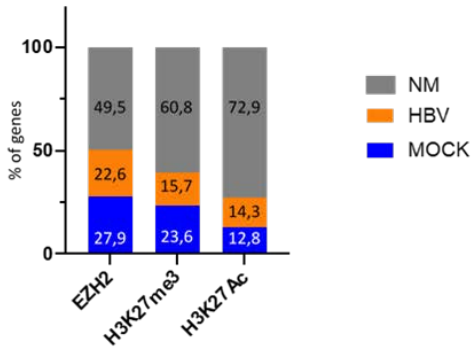

Figure . The percentage of EZH2 global recruitment and enrichment of H3K27 histone modification on the promoter regions

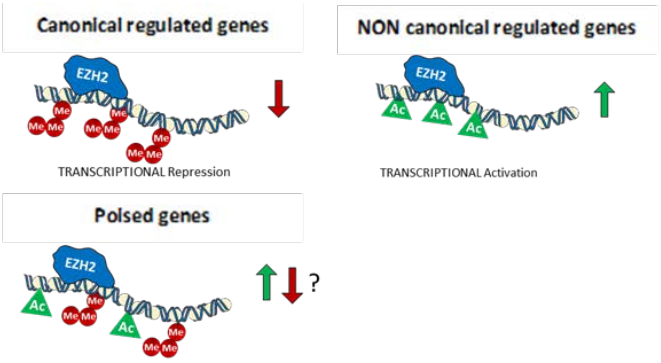

Figure. EZH2 functional roles in HepG2-NTCP cells (-/+HBV)

PO-280
Transcriptomic profiling of primary sclerosing cholangitis associated cholangiocarcinoma identifies novel genes of interest

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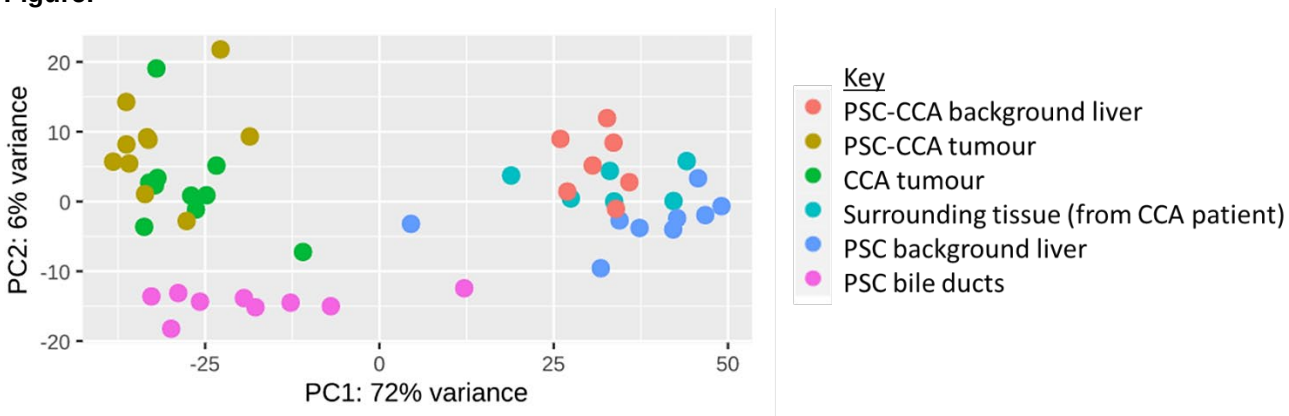
Background and aims: Primary sclerosing cholangitis (PSC) patients have a 20% lifetime risk of cholangiocarcinoma (CCA), equating to a 250-1500-fold higher risk than that of the average population. Despite this, PSC patients cannot be stratified for CCA risk, have limited treatment options, and face a poor prognosis. Current research on the degree of similarity between CCA in PSC patients and non-PSC patients is limited, and thus the applicability of CCA targeted therapeutic to PSC associated CCA (PSC-CCA) remains incompletely confirmed. This work aims to highlight novel differences in gene expression between PSC-CCA and CCA (non-PSC). This will provide novel mechanistic insight into disease development and progression in PSC-CCA, with the further aim of identifying biomarkers for targeted therapeutics.

Method: Patient samples, which were formalin fixed and paraffin embedded, were analysed for gene expression profiles via massive analysis of cDNA end (MACE) sequencing. Tissue was taken from CCA (n=10), PSC-CCA (n=10), PSC-CCA surrounding liver (n=7), PSC bile ducts (n=9), PSC surrounding liver (n=9) and CCA adjacent liver (n=6). Standard bioinformatic analysis was applied.

Results: Primarily, comparison of PSC-CCA and CCA tumours identified 2567 differentially expressed genes. Most significantly downregulated in PSC-CCA tumours included ribosomal processes when compared to both CCA tumours and PSC bile ducts. Specifically, ribosomal protein L10, L13a, L18, L23, L23a, L24, L36, S11, S14, S15, S27A, S5, S8, LP1 and LP2 were downregulated in PSC-CCA. Conversely, ion transport was significantly upregulated in PSC-CCA. When the background liver of non-malignant PSC and PSC-CCA patients were compared, 357 differentially expressed genes were identified. Apoptotic pathways were upregulated, and ribosomal processes downregulated in PSC-CCA background livers. The third comparison of PSC-CCA tumours to PSC bile ducts identified 1717 differentially expressed genes. Again, ribosomal processes were downregulated, alongside the complement cascade and extracellular matrix organisation in PSC-CCA tumours. Proliferation and migration were upregulated in PSC-CCA tumours.

Conclusion: This transcriptomic dataset identifies novel differences between PSC-CCA and CCA (non-PSC) and highlights a unique role for ribosomal proteins in PSC progression to CCA. The specificities of this are currently unclear, warranting further investigation.

Figure:



PO-284
Predictors of survival of 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. Patients unfit to receive any type of treatment are managed with best supportive care (BSC), and their median overall survival (OS) is around 3-6 months, although longer values may be observed in clinical practice. Aim of this study was to identify prognostic factors associated with longer survival in patients with HCC treated with BSC.

Method: We retrospectively evaluated the clinical characteristics of 916 patients, recorded in the Ita.Li.Ca. database, who had an indication for BSC. We analysed both patient and tumour characteristics to identify predictors of better OS.

Results: Median age was 71y and 75% of patients were male. Etiology included chronic viral infection (48.7%), alcohol use disorder (20.7%) and non-alcoholic steatohepatitis (4.1%). Approximately 50% of patients had a performance status 0-1 and were in Child-Pugh B class. Median MELD was 13. 60% of patients had a multifocal HCC with a median number of 2 lesions and a median size of 35 mm. 369 patients had vascular invasion. Median alpha-fetoprotein was 63.2 ng/ml. The median OS was 9 months (CI 7.7;10.2). No differences in terms of OS were observed considering the etiology of liver disease. Among comorbidities, heart disease was associated with lower OS (p=0.015). Abdominal pain (p<0.001), vomiting (p=0.014), fatigue (p<0.001), edema (p<0.001), jaundice (p=0.01), higher PS (p=0.01), and a worse liver function (p<0.001) were associated with shorter OS. Patients with multifocal HCC had a better OS (12 mo; CI 10-13.9) compared to those with monofocal HCC (8 months; CI 6.5-9.5) (p<0.001). Lack of vascular invasion was also associated with a better OS (14 months, CI 12.2-15.7, p<0.001). No significant differences were observed comparing patients with or without metastasis (p=0.310). Patients who had an active treatment before BSC had significantly longer OS than those for whom BSC was the only treatment (561 patients, p<0.001). Survival in BCLC-A patients was longer than in other stages. No differences in OS were found comparing BCLC-B and -C groups. Patients in BSC with a median OS longer than 6 months had more lesions (p=0.005), higher levels of albumin (p=0.003), lower bilirubin (p<0,001) and alpha-fetoprotein (0,001), and a lower median MELD (p<0.001). A weak association was found between survival shorter than 6 months and the presence of cirrhosis (p=0.012), alcohol consumption (p=0.046), heart disease (p=0.007), obesity (p=0.025), hypercholesterolemia (p=0.002), hypertriglyceridemia (p=0.0036) symptoms (p=0.02), vascular invasion (p<0.001), and non-multifocal HCC (p<0.001). Similar results were found comparing patients with a mOS longer or shorter than 12 months.

Conclusion: In a large series of patients with HCC in BSC we identified several clinical and tumour characteristics associated with the length of survival.

PO-286
Characterization of transforming growth factor (TGF)-beta signalling in sorafenib-resistant hepatocarcinoma cell lines.

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Background and aims: Transforming Growth Factor (TGF)-beta plays a dual role on Hepatocellular Carcinoma (HCC) malignant cells, behaving as a suppressor factor at early stages, but contributing to later tumour progression once cells escape from its cytostatic effects. Moreover, TGF-beta can modulate the response of the cells forming the tumour microenvironment, which may also contribute to HCC progression and drive immune evasion of cancer cells. Thus, targeting the TGF-beta pathway may constitute an effective strategy for HCC treatment, either alone or in combination. However, it could not benefit if HCC cells maintain the suppressor response to TGF-beta. Sorafenib has been used as first-line treatment in advanced HCC for the last decade, but most patients develop resistance mechanisms that compromise the response to treatment. We previously described that sorafenib sensitizes HCC cells to the apoptotic activity of TGF-beta. Therefore, we hypothesize that TGF-beta signalling maybe altered in patients who have developed resistance to sorafenib. Thus, the aim of this study is to evaluate the status of TGF-beta signalling and the effect of its inhibition on sorafenib-resistant HCC cells.

Method: Five HCC cell lines (Hep3B, HuH-7, PLC/PRF/5, SNU449 and HLF) with acquired resistance to sorafenib were used. Characterization of the status of the TGF-beta signalling pathway and the response to TGF-beta suppressor effects, as well as the evaluation of the impact of the TGF-beta inhibitor Galunisertib on these cells were conducted by Crystal violet cell viability assay, RT-qPCR, Western Blot and Immunofluorescence.

Results: Hep3B and HuH-7 cells, sensitive to TGF-beta suppressive effects, changed their response to this growth factor after development of resistance. The suppressive TGF-beta signalling was found totally or partially cancelled in sorafenib-resistant cells as ascertained by the levels of SMAD2 and SMAD3 phosphorylation. This response correlated with a dramatic reduction of the expression of TGF-beta Receptor II and I in Hep3B an HuH-7 cells, respectively. Altogether these alterations blunted the antiproliferative and proapoptotic effects of TGF-beta in Hep3B an HuH-7 resistant cells. In contrast, HCC cells with lower sensitivity to TGF-Beta, such as PLC/PRF/5, SNU449 and HLF, showed a preserved responsive TGF-beta axis after acquiring resistance to sorafenib. Treatment with TGF-beta inhibitor Galunisertib did not conduct to a relevant increase of cell proliferation in any of the sorafenib-resistant cells evaluated.

Conclusion: Our data indicate that, irrespective of the TGF-beta status in HCC parental cells, the use of a TGF-beta inhibitor would not promote cell proliferation in sorafenib-resistant cells. Therefore, patients who have developed resistance to sorafenib could benefit from this therapy, without having unwanted effects on tumour growth.

PO-287

PD1+ T-cells correlate with nerve fibre density as a prognostic biomarker in patients with resected perihilar cholangiocarcinoma.

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Email: lheij@ukaachen.de

Background and aims: Perihilar cholangiocarcinoma (pCCA) is a rare, but challenging and aggressive hepatobiliary malignancy arising from the bifurcation of the biliary system. Nerve fibre density (NFD) is presented as a novel prognostic biomarker in pCCA patients, however, the knowledge of this observation is limited. Nerve fibre invasion (NFI) can be seen as the opposite of NFD, while NFI shows cancer invading the nerve. NFD contains small nerve fibres without cancer invasion. We aim to explore the immune cell composition and expression of checkpoint regulators in these nerve fibre related phenotypes.

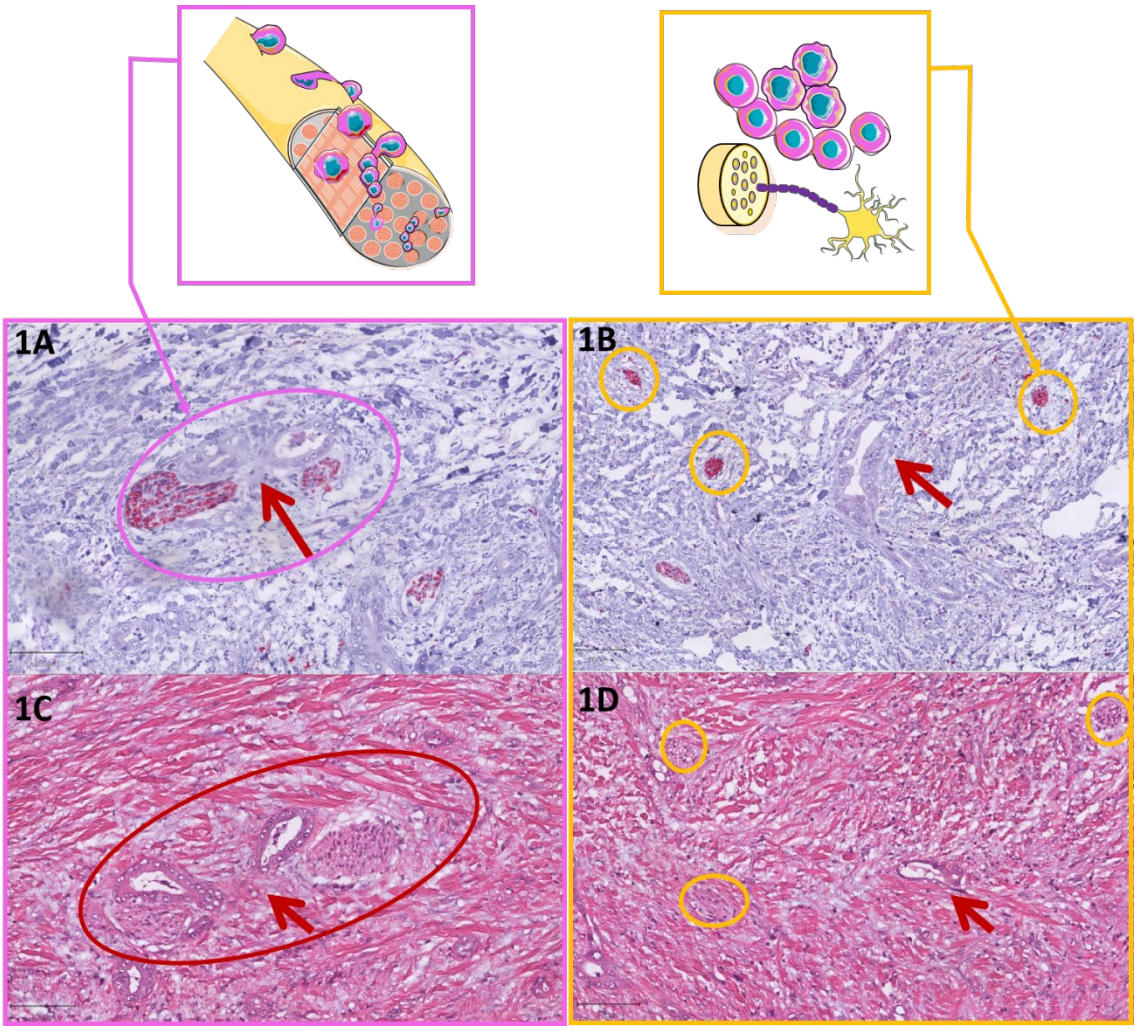
Method: We applied multiplex immunofluorescence (mIF) on 47 pCCA patients and investigated the immune cell composition and distribution in the tumour microenvironment (TME). We measured presence of immune cells and expression of their co-stimulatory and co-inhibitory markers. Extensive group comparison between patients with NFD and NFI was carried out and the association of overall survival (OS) was assessed.

Results: For high NFD patients CD68+ macrophages were enriched in the TME: the expression of CD68 was significantly higher in the tumour region of interest (ROI) compared with tumour free ROI in patients with NFI (p= 0,0325). The NFI ROI was measured with the highest CD68+ macrophage expression among 3 ROIs (NFI compared to tumour free p= 0,016 and to tumour p=0,034).

Comparison between patients with high NFD and NFI groups, the signals of co-expression of CD8+PD1+ as well as CD68+PD1+ were significantly higher in the high NFD group (p= 0,0436 and p= 0,0274, respectively). Overall survival (OS) among the high NFD patients, low NFD patients and NFI demonstrated a significant difference between the high NFD patients with a median OS of 50 months (95% CI:42-59) compared to a median of 29 months (95% CI 19-38) in patients with NFI (p= 0,004).

Conclusion: PD1+ T-cells correlate with high NFD as a prognostic biomarker, the biological pathway behind this needs to be investigated.

Figure:
Figure 1: The difference between Nerve Fibre Invasion (NFI) and Nerve Fibre Density (NFD). 1A NFI is defined as tumour cells invading the perineurium of the nerve. In the neuronal marker (PGP9.5) you see the nerve fibre in red (red arrow) surrounded and invaded by tumour cells and glandular structures. 1B NFD shows the presence of small nerve fibres, found in the tumour microenvironment (TME). The red arrow points to the tumour cells and the yellow circles mark the presence of small nerve fibres stained with the neuronal marker (PGP9.5). 1C Corresponding H&E staining of NFI. Nerve fibre invasion is recognisable for the pathologist. 1D H&E staining, the small nerve fibres are not detectable on this routine staining.



PO-288
Targeting of nuclear receptors and microRNA-21 ameliorates non-alcoholic fatty liver disease progression towards carcinogenesis in mice

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) has become a leading etiology underlying the development of hepatocellular carcinoma (HCC). In turn, ligands of the farnesoid X receptor (FXR) and G protein-coupled receptor 5 (TGR5) represent promising therapeutic targets in NAFLD. We aimed to elucidate whether INT-767, a dual FXR and TGR5 agonist, alone or in combination with miR-21 silencing, targeting peroxisome proliferator-activated receptor alpha (PPAR-α), could synergize in preventing NAFLD progression towards carcinogenesis.

Method: C57BL/6 mice were treated with a single intraperitoneal injection of diethylnitrosamine (DEN) and fed a high-fat choline-deficient (HFCD) diet for 14 weeks, and then treated with or without INT- 767, and/or with antagomiR-21 or a scrambled antagomir (control) for additional 10 weeks. Liver samples were collected and processed for histological and molecular assessments. Expression of genes and proteins involved in NAFLD and NAFLD-associated carcinogenesis were evaluated through qRT-PCR and immunoblotting.

Results: Animals fed the HFCD diet for 24 weeks developed preneoplastic nodules (3-5 nodules; n=6 out of 13 mice), whereas animals treated with INT-767 and/or antagomiR-21 presented with less and smaller macroscopic preneoplastic nodules (0-2 nodules). Profiling of liver tumour-associated genes using qRT-PCR array plates showed that, out of 168 genes, 98 genes were modulated by at least 25% in the liver of either the HFCD-fed animals, comparing with control mice; or the INT-767 and/or antagomiR-21 HFCD animals, comparing with HFCD-fed mice. Pathway enrichment and gene ontology analysis, followed by qRT-PCR validation, showed that cancer-related genes found increased in HFCD-fed mice, and which included Cdk1, Fstl1 and Ccnb2, were particularly downregulated in antagomiR-21-treated animals. Finally, animals fed the HFCD diet displayed increased hepatic and visceral adipose tissue Fabp4 mRNA expression, comparing with those concomitantly treated with INT-767 and/or antagomir-21. Of note, NAFLD patients expressing high hepatic levels of FABP4 are more likely to experience disease progression, while upregulation of hepatic FABP4 expression has been associated to obesity-related HCC in mice.

Conclusion: Dual agonism of FXR and TGR5 by INT-767 and concomitant miR-21 inhibition regulate multiple overlapping and non-overlapping cellular and molecular pathways which are crucial for amelioration of NAFLD-associated carcinogenesis.

PO-289
Lipid droplets are mechanical stresses in non-alcoholic fatty liver disease

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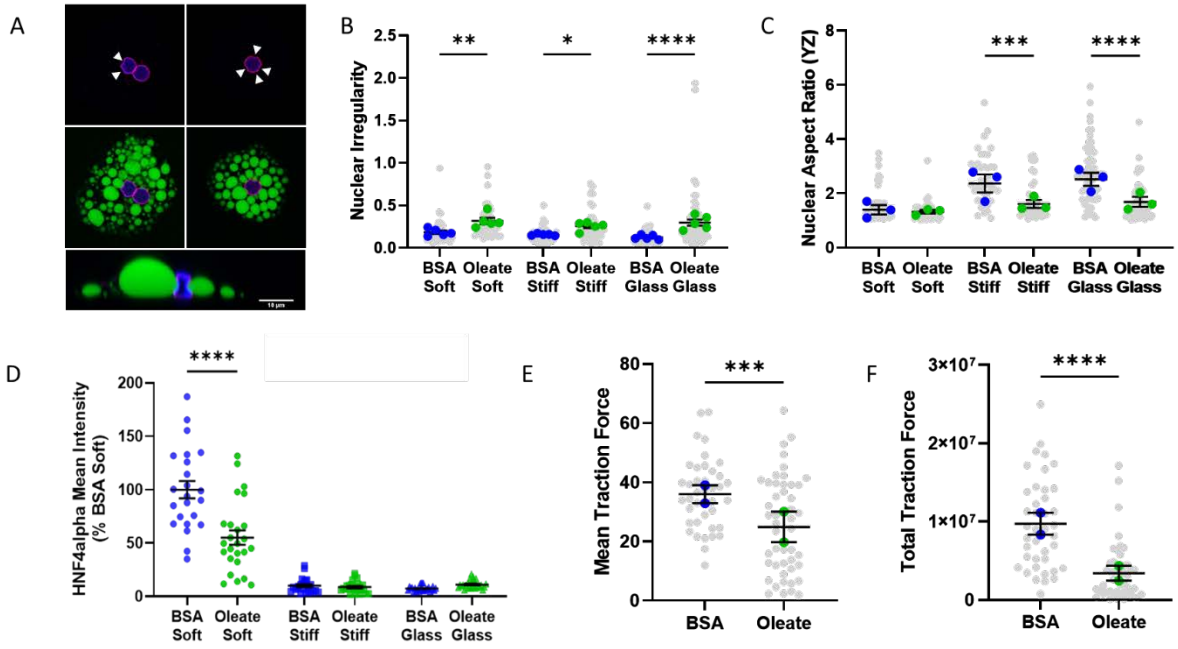
Background and aims: Hepatocellular carcinoma (HCC) develops in the context of chronic liver disease, with ~80% occurring in stiff cirrhotic livers. Increased tissue stiffness correlates to increased risk of HCC and poorer clinical outcomes, suggesting that mechanical stress contributes to the initiation and progression of cancer. However, HCC can occur in soft livers with non-alcoholic fatty liver disease (NAFLD). We hypothesize that lipid droplets (LDs) in NAFLD are an intracellular mechanical stress, disrupting the cytoskeleton, and directly deforming the nucleus.

Methods: Primary human hepatocytes (PHHs) were cultured on glass, 500Pa and 10kPa polyacrylamide gels, coated with 0.1 mg/mL type I collagen. After 48hrs, cells were switched to serum-free media supplemented with 400nM oleate for an additional 48hrs. Cytoskeletal contraction (using 5uM latrunculin, 10uM nocodazole, or 5uM blebbistatin) was inhibited for 4hrs after lipid loading. Cells were stained using rhodamine-phalloidin, HNF-4a antibody or α-tubulin antibody and imaged by confocal microscopy. Staining intensity and fibre organization were quantified in ImageJ, while nuclear deformation was quantified using a custom MATLAB program. Traction force microscopy was also performed.

Results: On all stiffnesses, the nuclei of lipid-loaded cells have lower volume and spread area than controls. Cell volume remains unchanged however, indicating that hepatocytes are not expanding to accommodate lipid volume. Nuclei of lipid-treated cells are significantly more deformed than controls and LDs visibly deform nuclei (A, B). However, the nuclei of oleate-treated hepatocytes were taller than untreated cells (C), suggesting that lipid droplets resist compression. Actin fibres in oleate-treated cells exhibit less alignment in response to stiffness than controls, and branch more often as they are interrupted by LDs. Traction force measurements show a significant decrease in mean, and total traction force with lipid loading, and correlated with lipid density (E, F). LDs indent the nucleus even in the absence of an intact cytoskeletal network, suggesting that droplets directly exert mechanical force. HNF-4α is reduced by stiffness and lipid loading, indicating a LD-associated nuclear stress (D).

Conclusions: Lipid-loading of hepatocytes leads to radial deformation of the nucleus, while resisting compression by the actin cortex. Inhibition of the cytoskeleton has little impact on hepatocyte morphology in oleate-treated cells, suggesting that LDs directly indent the nucleus and resist cytoskeletal-driven morphological changes. This is consistent with evidence that LDs disrupt the cell cytoskeleton and reduce traction-forces. Together, these results suggest that intracellular LDs are mechanical elements that directly cause nuclear stress and could promote dedifferentiation in NAFLD.

Figure:


A
B
Nuclear Irregularity
**
*
****
BSA Soft
Oleate Soft
BSA Stiff
Oleate Stiff
BSA Glass
Oleate Glass
C
Nuclear Aspect Ratio (YZ)
***
****
D
HNF4alpha Mean Intensity (% BSA Soft)
E
Mean Traction Force
BSA
Oleate
F
Total Traction Force

PO-290
SerpinB3/4 expression is associated with poor prognosis in patients with cholangiocarcinoma

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Background and aims: Cholangiocarcinoma (CCA) is characterized by a very poor outcome and limited prognostic markers are currently available for this dismal tumour. The protease inhibitor SerpinB3 has been recently identified as a critical mediator of malignant phenotype in different tumours.
The aim of the study was to analyse tissue and serum expression of SerpinB3/4 in human CCA, in relation to clinical outcome.

Method: SerpinB3/4 was evaluated by tissue microarrays (TMAs) in 123 surgically resected CCAs, that were dichotomized into SerpinB3/4 high (2+/3+) *versus* SerpinB3/4 low (0/1+) groups. ELISA assays to detect free and IgM linked forms of this serpin in serum were carried out in additional 188 patients with CCA. Overall Survival was analysed in relation to SerpinB3/4 expression and was estimated with Kaplan-Meier methods. Univariate and multivariate Cox models were used to evaluate independent variables associated with survival.

Results: Fifteen tumours (12.2%) showed high levels of SerpinB3/4 (TMA score 2+/3+). Patients with high SerpinB3/4 scores presented more frequently advanced TNM Stage (III/IV:64.3% vs. 31.3%, p=0.031), and had higher serum CA 19-9 levels (328 vs. 53 kU/L, p=0.001). Patients with high SerpinB3/4 scores had lower overall survival, independently of CCA subclass (iCCA: median 1.1 vs 2.4 years; p = 0.0007; eCCA: median 0.8 vs 2.2 years; p = 0.011). In a multivariate analysis, vascular invasion (p=0.027) and SerpinB3/4 score (p=0.0016) were independently associated with mortality. Patients who were positive for either free or IgM -linked SerpinB3/4 in serum showed poorer survival (1 vs 2.4 years for free SerpinB3/4, p 0.015; and 1 vs 2.6 years, p 0.0026 for SerpinB3/4-IgM).

Conclusion: High levels of tissue and serum expression of SerpinB3/4 in CCA are strongly associated with poor outcome after surgery, regardless of tumour subclass.

PO-293
Anticancer activity of novel vicinal diaryl isoxazole compounds in hepatocellular carcinoma

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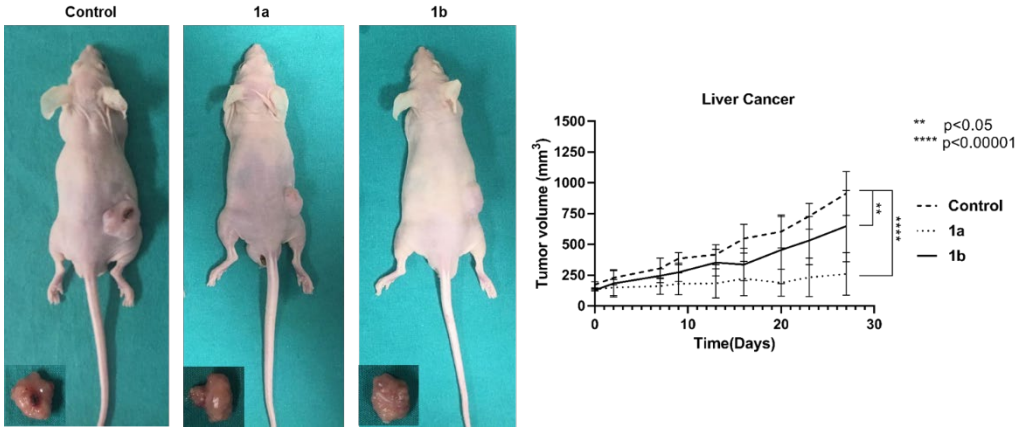
Background and Aims Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and 2nd lethal cancer in the world. The global burden of HCC is expected to increase in the next decade due to the increase in obesity and related fatty liver disease cases in the world. Because of hyperactivation of many critical cell survival signaling pathways, HCC is highly resistant for conventional chemotherapies and targeted agents extend the patient's survival for only six months. It is crucial to design and develop novel therapeutics against HCC. Here, we investigated the possible cytotoxic bioactivities of novel vicinal diaryl isoxazole compound on HCC.

Method: Bioactivities of compounds were identified with NCI-SRB assay and RT-CES analysis. PI staining was performed for cell cycle using flow cytometry. Apoptotic cells were detected through both fluorescence microscopy and by flow cytometry. Changes in transcriptome level was controlled by multiplexed analysis of PanCancer panel for 770 cancer genes. Western blot analysis was done to assess the protein level of cell cycle and apoptosis related signaling pathways. *In vivo* tumour xenograft assay was performed to test the anti-cancer activity of the potent compounds.

Results: Among all 48 compounds tested two of them (**1a** and **1b**) showed very promising cytotoxic activities and were selected to be further biological testing against HCC cell lines. Both **1a** and **1b** compounds were identified as significant anti-cancer agents upon liver cancer cells with lower cytotoxic doses (<3 mikro molar). Time-and dose-dependent growth inhibition upon treatment with **1a** and **1b** was observed due to cell cycle arrest in S and G2/M phases and apoptosis.The cell cycle pathway and related genes were the most differentiated pathway and genes by analysis of cancer panel. The expressions of S and G2/M cyclins were also changed in protein level with treatment. Furthermore, upon treatment with both **1a** and **1b**, the tumour size decreased in nude mice significantly.

Conclusion: Altogether, the anti-tumour effects of the compound **1a** and **1b** approve that these small molecules can be considered as eventual anti-cancer agents for liver cancer.
This study was supported by a Research Grant from TUBİTAK (#215S015).

Figure:


Control
1a
1b
Liver Cancer
** p<0.05
**** p<0.00001
Tumor volume (mm3)
1500
1250
1000
750
500
250
0
0
10
20
30
Time(Days)
Control
1a
1b

PO-295

Serum IL-8 is associated with altered neutrophil phenotype and poorer survival in patients with hepatocellular carcinoma

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Background and aims: As deaths from hepatocellular carcinoma (HCC) rise, understanding the tumour-immune axis is key. Elevated circulating neutrophils associate with poor survival. Understanding the pathophysiological mechanisms underpinning rising neutrophil numbers and changes in phenotype may aid novel therapeutic strategies. We explored serum cytokine associations with tumour stage, patient outcome and the phenotype of circulating neutrophils.

Method: Serum from a pilot cohort of patients (chronic liver disease (CLD) no cirrhosis n=7; CLD with cirrhosis n=6; HCC no cirrhosis n=10; HCC with cirrhosis n=12) was subjected to a cytokine 65-Plex panel (Eve Technologies). Subsequent ELISA validation was in 129 patients. All patients had preserved liver function. 49 had liver biopsy tissue for CD66B neutrophil immunohistochemistry, 17 had cytospin nuclear maturity assays and 40 had neutrophil FACS analyses for CD11b,CD15,CD16,c-MET,PD-L1,TIM-3,CD10,CXCR4 and CD62L.

Results: Of cytokines assayed in the pilot study, elevations in IL-8 (pg/ml) were most dramatically and significantly associated with HCC (CLD no cirrhosis 5.1+/-1.6,n=7; CLD with cirrhosis 12.1+/-4.6, n=6; HCC no cirrhosis 43.7+/-21.9,n=10; HCC with cirrhosis 25.1+/-5.2, n=12; p<0.0001). By ELISA (n=123 patients with HCC), there were highly significant increases in IL-8 with TNM stage (p=0.001) and portal vein thrombosis (no PVT 64.4+/-9.2; with PVT 156.2+/-39.2; p<0.0001). IL-8 was negatively associated with albumin (Spearman Rho -0.250, p=0.004). The mean survival of patients with serum IL-8 >median (43.4) was 12.3 months compared to 39.28 months for IL-8 <median (Figure 1; p=0.0001). In a multivariate Cox Regression analysis including tumour size, vascular invasion, albumin, bilirubin and ECOG performance status, serum IL-8 was independently associated with poorer survival. Serum IL-8 was weakly associated with circulating neutrophil numbers, but strongly associated with higher % banded immature neutrophils (Spearman Rho 0.653, p=0.011) and negatively with % hypersegmented neutrophils (-0.644, p=0.013). There were also significant increases in neutrophil CXCR4 and c-MET expression detected by FACS. In tissues, serum IL-8 was positively associated with rising peri-tumour/tumour CD66B+ neutrophil ratio (Spearman 0.450, p=0.047).

Conclusion: In patients with HCC, Elevated IL-8 was associated with advanced tumour stage, vascular invasion, deteriorating liver function and independently with poorer survival. IL-8 is a known neutrophil chemotactic and stimulating factor and potent promoter of angiogenesis. Here IL-8 was associated with elevated immature circulating neutrophils and changes in neutrophil phenotype, as well as altered distribution of neutrophils in tumour tissues. We suggest that these are mechanisms promoting the development and progression of HCC.

Figure:

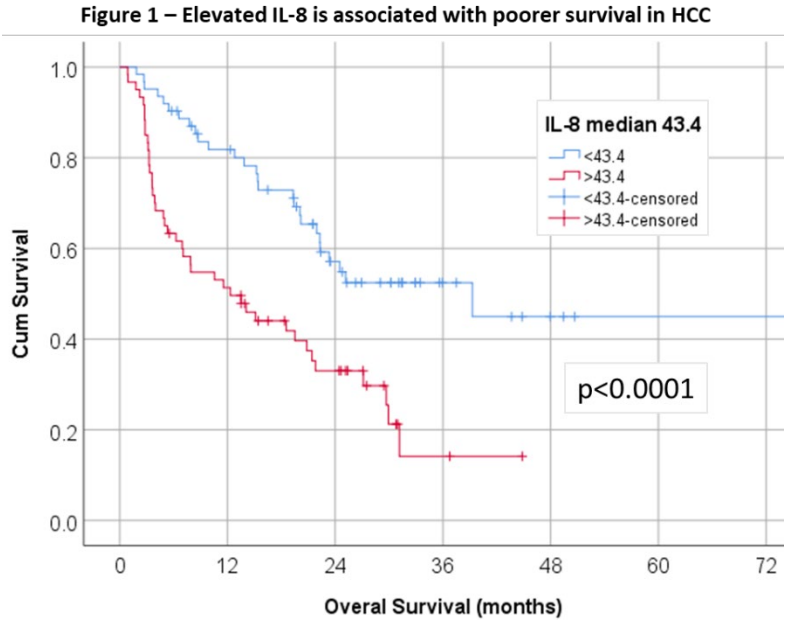
Figure 1 – Elevated IL-8 is associated with poorer survival in HCC
IL-8 median 43.4
<43.4
>43.4
<43.4-censored
>43.4-censored
p<0.0001
Cum Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
12
24
36
48
60
72
Overal Survival (months)

PO-296
In vitro effects of magnetic hyperthermia via a nanomodified polymer stent for cholangiocarcinoma treatment

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Email: aroeth@ukaachen.de

Background and aims: Patients with perihilar cholangiocarcinoma often suffer from biliary stasis with resulting cholangitis, especially in the palliative situation. The current gold standard treatment is the placement of an endoscopic stent that needs to be exchanged regularly. Previously, we developed hybrid stents consisting of nanomodified filaments that are able to heat the tissue to a temperature of 42-45°C by applying an external magnetic field. As tumour tissue reacts more sensitive towards hyperthermia, this hybrid stent may hold the biliary duct open mechanically as well as impair tumour growth by magnetic hyperthermia. Here, we aimed at demonstrating the effects of magnetic hyperthermia treatment in an in vitro model.

Method: Toxicity experiments on the cholangiocarcinoma cell line TFK-1 and the murine fibroblast cell line L929 were performed via a viability test with Cell Titer Glo for filaments with different concentration of magnetic nanoparticles. Using these filaments, grids were fabricated and the in vitro effects of magnetic hyperthermia on TFK-1 cells in a 3-dimendional environment of collagen were investigated. The “grid plus alternating magnetic field” group was compared to the control groups: the “grid alone” group, the “alternating magnetic field” group and a DMSO-group as negative control. The biological effects were examined by immunohistochemistry of different antibodies as well as multiplex imaging with TissueFAXS.

Results: The toxicity tests revealed no toxicity for the filaments. We were able to generate heat in the 3D-model of TFK-1 cells up to 43°C. The viability tests demonstrated a significant reduction up to 35 % in the “grid plus alternating magnetic field” group. Immunohistochemistry as well as TissueFAXS-analysis of the cells revealed an enhancement of heatshock proteins 70 and 90 as well as DNA damage (γ-H2AX) and apoptosis (cleaved caspase-3)

Conclusion: Treatment of perihilar cholangiocarcinomas with magnetic hyperthermia using a hybrid stent is a promising alternative to standard treatment and is able to impair tumour growth.

PO-300
Genes modulating liver fat accumulation and lipogenesis predict development of hepatocellular carcinoma among direct antiviral agents treated cirrhotics C with and without viral clearance

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Background and aims: Genetic risk score (GRS) is a polygenic scoring system that estimates the predisposition to accumulate liver fat combining PNPLA3 (rs738409), TM6SF2 (rs58542926), MBOAT7 (rs641738) and GCKR (rs1260326) polymorphisms. GRS score was related to the probability to de novo hepatocellular carcinoma (HCC) development in HCV cirrhotic patients, treated with direct antiviral agents (DAA). In this setting, it is suggested that HSD17B13: TA variant (rs72613567) exhibits a protective role on fibrosis development and hepatocarcinogenesis. Our aim was to evaluate if HSD17B13 variant together with achievement of viral clearance (SVR) affected the efficay of GRS in HCC prediction.

Method: 328 HCC free cirrhotic patients were included. Diagnosis of cirrhosis was based on transient hepatic elastography (liver stiffnes ≥ 12.5 kPa) or on clinical diagnosis of liver cirrhosis. Genomic DNA was extracted from whole blood. PNPLA3 and HSD17B13 genotypes were determined using restriction fragment length polymorphism technique. TaqMan® SNP (Life Technologies) genotyping test was used to identify TM6SF2, MBOAT7 and GCKR genotypes. GRS score was calculated as previously described by Degasperi et al, Hepatology 2020.

Results: At the end of the follow-up (median, 7.5 months), n= 21 patients were diagnosed have de novo HCC (Group A). Among them, 4 patients were relapsers and 1 was a dropout, while in HCC-free patients group (Group B, n= 307), 8 relapsers (p= 0.001) were observed. The SVR (intention-to-treat) was of 76% in Group A vs. 97% in Group B. In Group A, patients were mainly male, with more advanced liver disease. None of the genes included in the GRS was individually associated with de novo HCC. In a Cox proportional hazards model, a GRS value ≥ 0.457 (75th percentile) predicted the onset of HCC regardless of gender, diabetes, albumin, INR and FIB4 (HR 2.89, C.I.1.19 - 7.07, p 0.02). The goodness of the model was improved by adding SVR achievement and presence of HSD17B13 variant; in particular, male gender (HR 6.75, C.I.1.62 - 28.2, p 0.009), GRS ≥ 0.457 (HR 4.24, C.I. 1.59 – 11.33, p 0.004), carriage of the splice variant HSD17B13: TA (HR 0.24, C.I. 0.07 - 0.75, p 0.015) and failure to achieve SVR (HR 7.7, C.I. 2.38 - 25, p 0.001), were independent predictors of de novo HCC.

Conclusion: The study results confirmed that genes modulating lipogenesis and liver fat are important risk factors for the development of HCC in HCV cirrhotic patients treated with DAA.

PO-302
Comparison of therapeutic outcomes of liver resection and transarterial chemoembolization in multifocal HCC: a propensity score-matched analysis

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Background and aims: Patients with multifocal hepatocellular carcinoma (HCC), preserved liver function, without cancer-related symptoms (PS 0), vascular invasion or extrahepatic spread represent a heterogeneous group. Although transarterial chemoembolization (TACE) is the most frequently employed treatment in this group of patients, previous data have indicated that liver resection (LR) could be a safe and effective procedure for multifocal HCC. Nevertheless, it is still debated whether better outcomes are achieved after surgery as compared to those obtained with TACE.

Method: We prospectively enrolled 58 patients with multifocal HCC who underwent a first procedure of LR (=25) or TACE (=33) between May 2011 and March 2021. For each patient, information regarding demographic and clinical variables was collected. A propensity score matching was used to adjust the baseline differences between patients undergoing LR and the TACE group (number and diameter of lesions, presence of cirrhosis, AFP values, and MELD score). TACE and LR were compared in terms of overall survival, disease-free survival and development of short-term (<7 days) complications.

Results: Median age was 68 and 70 years for LR and TACE, respectively. In both groups almost all patients were male and chronic viral infection was the most frequent etiology. The median MELD in the LR and TACE groups was 8 and 9, respectively and most patients had cirrhosis. Regarding tumour features the median number of lesions was 3, median size 65 mm and median alphafetoprotein 16 ng/ml. The development of short-term complication was significantly higher in patients with LR (49%) than in those subjected to TACE (8%, p=0.015). The majority of complication within the LR group were classified as Clavien-Dindo I-II. In contrast, disease-free susrvival was significantly longer in resected patients (18.8 vs 4.8 months; p=0.004). Although a trend toward better overall survival was observed in patients undergoing TACE (27.9 months) compared with the LR group (22,4 months), the difference was not statistically significant.

Conclusion: In patients with multifocal HCC, LR confers an advantage in terms of disease-free survival compared with patients who underwent TACE, but this does not reflects into differences in terms of overall survival.

PO-306
Modulation of the cholangiocarcinoma stem-like compartment by monounsaturated fatty acids

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Background and aims: Identification of the molecular features of CCA may be helpful in designing new therapeutic approaches. Cancer cells are exposed to a metabolically challenging environment with scarce availability of nutrients, and alterations in lipid metabolism may affect the response of tumour cells to drugs. We hypothesize that fatty acids (FA) modulate the biology of CCA cells and the development of stemness features.

Method: CCA cells (HuCCT-1 OR CCLP1) were treated with monounsaturated FA (132mcM oleic or 100mcM palmitoleic acid). Responsiveness of CCA cells to cytotoxic drugs was tested with crystal violet staining. Epithelial-mesenchymal transition program, stem-like markers, ABC transporters and metabolic markers, were tested with real-time PCR. 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 analysed in 104 patients with cholangiocarcinoma sub-grouped based on fatty acid synthase (FASN) expression. NSG mice were injected with spheres obtained from CCLP1 cells and treated for four weeks with the FASN inhibitor orlistat (240mg/Kg).

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 before treatment with chemotherapeutic agents made CCA cells less sensitive to their toxic effects, 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. In a series of CCA patients, the expression of FASN correlated with OS. FASN inhibition by orlistat decreased cell proliferation and CSC or EMT markers. In a xenograft model of CCA, tumour volume of mice treated with orlistat was significantly lower than in control mice.

Conclusion: Exposure of CCA cells to FA increases growth, invasiveness and resistance to antineoplastic drugs, and modulates stem-like features and self-renewal abilities. In the CCA stem-like subset, several key genes involved in FA synthesis and transport were upregulated, and FASN inhibition decreased cell proliferation and downregulated CSC markers. and FASN expression levels correlate with survival in patients with CCA. These data suggest that lipid metabolism could be a new potential target to affect CCA progression, especially in CSCs subset.

PO-307
Potential role of soluble triggering receptor expressed on myeloid cells 2 in risk stratification of patients with hepatocellular carcinoma

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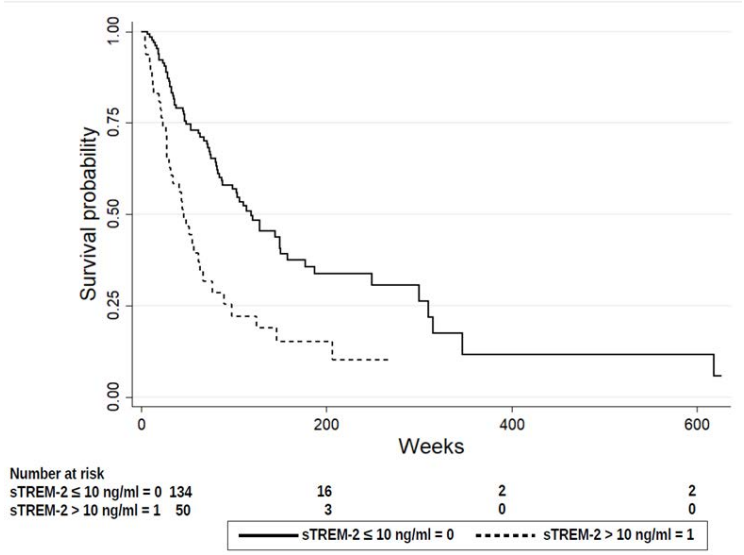
Background and aims: Triggering receptor expressed on myeloid cells 2 (TREM-2) is a transmembrane receptor of the immunoglobulin superfamily that has been recently studied in many diseases including liver cancer. Nevertheless, its role is still unclear and there are no studies that have evaluated its soluble form (sTREM-2) as a biomarker. Thus, we aimed to explore the prognostic value of serum sTREM-2 in hepatocellular carcinoma (HCC).

Method: An observational cross-sectional study was performed on 184 HCC patients of any etiology, in stage A, B or C of the BCLC classification, enrolled between 2005 to 2021. Diagnosis of HCC was established according to the AASLD and the EASL guideline criteria. All patients underwent genotyping of TREM-2 polymorphism (rs6918289) trough polymerase chain reaction DNA amplification (PCR DNA) and serum sTREM-2 levels were quantified by using an enzyme-linked immunosorbent assay (ELISA).

Results: The median of sTREM-2 was 7.4 ng/ml [5.7 - 10.4 ng/ml]. sTREM-2 levels had a week correlation with age (p = 0.024) and stronger with AST (p = 0.001). From the univariate analysis, an association with the BCLC stage emerged: more advanced stages corresponded to higher values of sTREM-2 (p = 0.001). The allele frequency of rs6918289 polymorphism was 92% (339/368) for the ancestral allele (G) and 8% (29/368) for the variant allele (T). The distribution by genotype did not differ from what was expected according to the Hardy-Weinberg equilibrium (p = 0.246). At the end of a maximum period of observation (11.9 years), the overall mortality was 58.15% (107/184), with a median survival time of 86 weeks [31-287]. Survival analysis showed that the increase in sTREM-2 levels over the 75th percentile threshold (cutoff = 10 ng/ml) was significantly associated with a reduction in overall survival (log rank test with p < 0.0001, Figure). The Cox proportional hazards model built having as predictors sTREM-2, sex, age, presence/absence of cirrhosis/HCV infection and BCLC stage, confirmed that serum concentration of sTREM-2 > 10 ng/ml is an independent predictor of mortality (HR 1.88, CI 1.22 - 2.88, p = 0.004).

Conclusion: The present study demonstrated the potential negative prognostic role of sTREM-2 on overall HCC survival. However, future prospective studies are necessary to elucidate whether the systematic assessment of sTREM-2 concentrations could be useful to predict the survival in HCC patients.

Figure:


Survival probability
0.00
0.25
0.50
0.75
1.00
0
200
400
600
Weeks
Number at risk
sTREM-2 ≤ 10 ng/ml = 0 134 16 2 2
sTREM-2 > 10 ng/ml = 1 50 3 0 0
sTREM-2 ≤ 10 ng/ml = 0
sTREM-2 > 10 ng/ml = 1

PO-308
Liver transplantation for combined hepatocellular-cholangiocarcinoma: An analysis of the European Liver Transplant Registry

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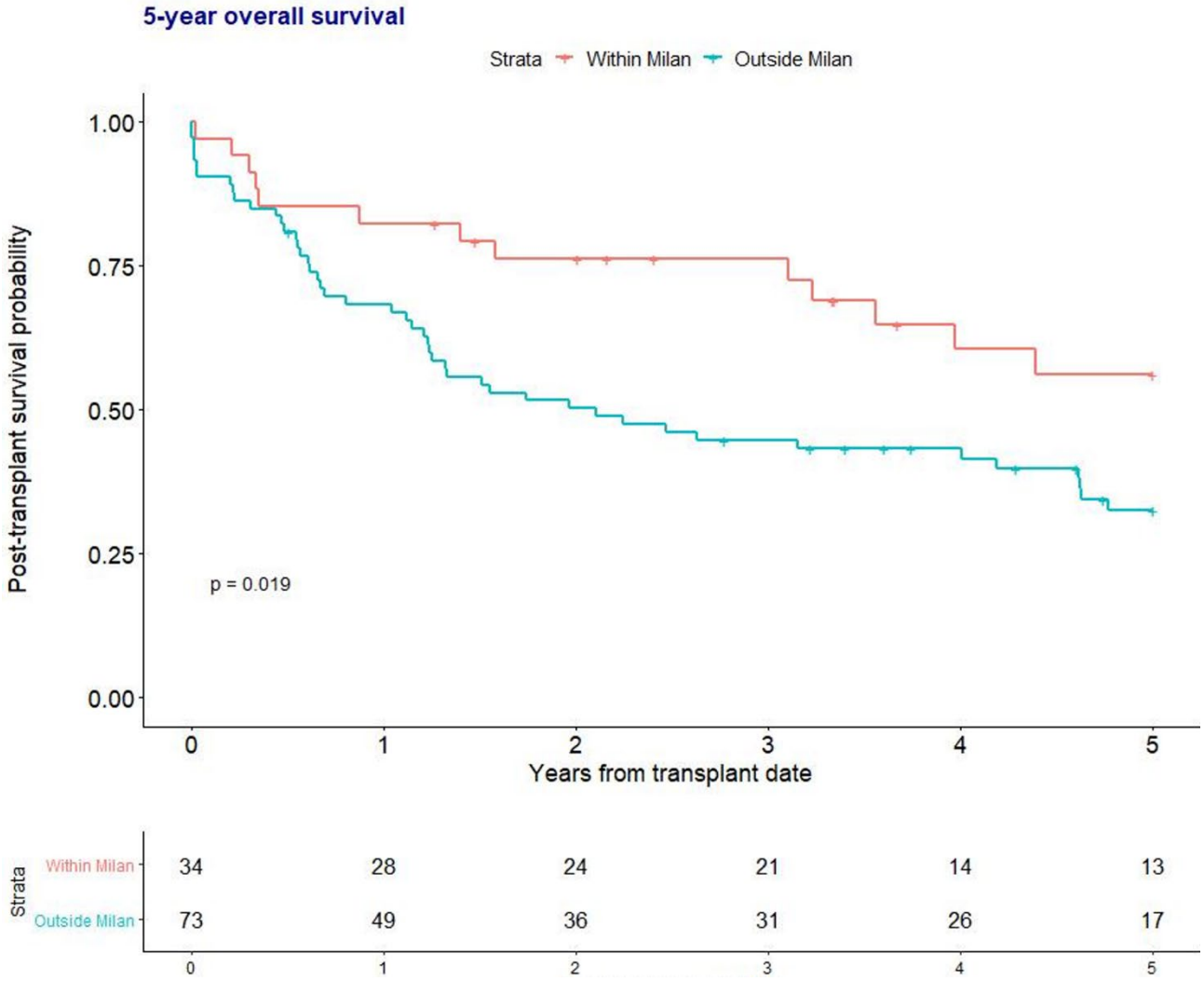
Background and aims: Data on combined hepatocellular-cholangiocarcinoma (cHCC-CCA) demographics and their long-term outcomes after liver transplantation (LT) are limited. We sought to map the characteristics of cHCC-CCA patients and identify patient subgroups that demonstrate favorable long-term outcomes after LT for cHCC-CCA by analysing data from the European Liver Transplant Registry (ELTR).

Method: ELTR-centres with cHCC-CCA patients transplanted before Jul-2021 were identified and contacted for study participation. Patients without diagnosis confirmation or follow-up data were excluded. Overall survival (OS) and recurrence-free survival (RFS) curves were estimated using the Kaplan-Meier method and compared by the log-rank test. A cumulative incidence approach was used to assess tumour recurrence, considering death without recurrence as a competing event.

Results: A total of 115 patients with cHCC-CCA were transplanted in 22 centres. Most (97%) were transplanted within the last 20 years. The majority were male (83%) with a median age of 59 years. Seventy-nine (69%) patients were listed as HCC, while only 4 patients were diagnosed with cHCC-CCA pre-operatively (3%). The most common underlying liver diseases were alcoholic liver disease (35%), hepatitis C virus (25%), and hepatitis B virus (17%). Median tumour number at listing was two (IQR 2-2), with a median maximum size of 28mm (IQR 20-41mm). About two-thirds of the patients were within Milan criteria at listing and at transplant. Median MELD at transplant was 11 (IQR 9-16). Tumour markers pre-LT were on median: AFP 15 (IQR 5-67), CA19.9 26 (IQR 13-58), CEA 3.3 (2.0-5.3). Before LT, 56% received TACE, 17% liver resection, and 15 % RFA. Explant pathology showed a median tumour number of two (IQR 1-4), maximum size of 32mm (23-50mm), with vascular invasion present in 40%. As a result, two-thirds were classified as outside Milan criteria. Cirrhosis was present in 94% of explanted livers. Most tumours were moderately (41%) or poorly (33%) differentiated. Recurrence occurred in 50 patients and most often included extrahepatic sites (77%). OS at 1, 3, 5-years was 70%, 51%, 37%, where RFS was 60%, 40%, 32%. Cumulative incidence of recurrence was 22%, 39%, and 42% at 1, 3, and 5-years. Within vs. outside Milan, based on explant pathology, showed an OS of 82%, 76%, 56% within Milan vs. 68%, 45%, 32% outside Milan at 1, 3, and 5 years ($p = 0.02$), where RFS was 82%, 62%, 54% within Milan and 53%, 33%, 25% outside Milan ($p < 0.01$). Cumulative incidence of recurrence was 8%, 23%, 23% within Milan vs. 29%, 46%, 51% outside Milan at 1, 3, and 5-years ($p < 0.01$).

Conclusion: Most cHCC-CCA patients were male and diagnosed post-LT after being listed for HCC. Alcoholic liver disease was the most common underlying liver disease. Patients within Milan criteria demonstrated acceptable 5-years OS and recurrence rates, superior to those outside Milan.

Figure:


5-year overall survival
Strata
Within Milan
Outside Milan
Post-transplant survival probability
1.00
0.75
0.50
0.25
0.00
p = 0.019
0
1
2
3
4
5
Years from transplant date
Strata
Within Milan
Outside Milan
34
28
24
21
14
13
73
49
36
31
26
17
0
1
2
3
4
5

PO-309
Cross-talk between MerTK-expressing stromal cells and cholangiocarcinoma

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Background and aims: A typical feature of cholangiocarcinoma (CCA) is a dense stromal reaction populated by fibrogenic myofibroblasts and immune cells, creating a complex tumour microenvironment where malignant cells survive and proliferate. Cancer stem cells (CSCs) have been proposed as a driving force of tumour initiation, dissemination and drug-resistance in many solid tumours, including cholangiocarcinoma (CCA). Increasing evidence indicates that myeloid-epithelial-reproductive tyrosine kinase (MerTK) is highly expressed by a macrophage subset defined as M2c. The present study aims to investigate whether signals generated by MerTK-expressing macrophages modulate the biology of CCA.

Method: 3D-tumour sphere cultures enriched in CSC were generated from intrahepatic CCA cell lines (HuCCT-1 and CCLP-1). Circulating monocytes were differentiated into M2c macrophages in vitro. Recombinant Gas-6, a MerTK ligand, was used to activate this receptor. MERTK mRNA expression in human CCA tissues was also analysed.

Results: In CCA cell lines cultured with conditioned medium from Gas-6-stimulated M2c macrophages, cell survival, invasion, sphere-forming efficiency and drug resistance were significantly increased. These effects were reduced following macrophage pre-treatment with the MerTK inhibitor, UNC2025. Analysis of the transcriptome of laser-captured, micro-dissected epithelium and stroma from 23 CCA patients showed that MerTK mRNA expression is significantly higher in intratumoural stroma. Single-cell RNA sequencing of CD45+ sorted cells from paired non-tumoural and tumoural specimens from iCCA patients (n=6) defined eleven clusters characterized by their gene expression profiles. A further reclustering of myeloid cells showed MerTK expression in Kupffer cells, lipid macrophages, TREM2+ macrophages, and non-classical monocytes.

Conclusion: These data suggest a cross-talk between MerTK-expressing cells in the stroma and CCA cells, to induce increased malignant features.

PO-312
Performance of Toronto HCC risk index and aMAP score in predicting HCC development in patient with cirrhosis

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Background and aims: Hepatocellular carcinoma (HCC) is a life-threatening complication of cirrhosis which requires periodic and systematic screening. The Toronto HCC risk index (THRI) and the age-male-ALBI-platelets (aMAP) score are recently proposed simple scores for the prediction of the development of HCC in chronic liver disease. Our objective was to evaluate the performance of these scores in predicting HCC development in patients with cirrhosis.

Method: We performed a retrospective analysis of data from consecutive cirrhotic patients, followed in our department, recruited from January 2010 to December 2019. The THRI was calculated at the first admission for all patients. Referring to the Canadian study from the University of Toronto, the patients were then classified according to the level of risk of HCC: group 1 (G1) of low risk (<120), group 2 (G2) of intermediate risk (between 120 and 240) and high risk group 3 (G3) (> 240). The aMAP score was calculated using the following formula:
({0.06×age+0.89×sex(Male:1,Female:0)+0.48×[(log10bilirubin×0.66)+(albumin×−0.085)]−0.01×platelets}+7.4)/14.77×100.

A aMAP score <50 and >60 points identified patients at distinctly low and high risks respectively.
Results: A total of 224 patients were included with an average age of 61.02 ±13.2 years and a sex-ratio of 1.6. The main etiology of cirrhosis was viral infection C (32.1%) followed by viral infection B (22.8%) and non-alcoholic steatohepatitis (21.4%). Sixty-one patients had developed a HCC (27,85%) during follow-up. Patients who developed HCC had a higher THRI score than those who did not develop HCC (278.92 ± 62.19 vs 226.23 ± 81.72, respectively, p <0.001). At a cutoff of 226, THRI had a sensitivity and specificity in predicting degeneration of 80.3% and 48% respectively. The patients were classified according to the level of risk: 7.3% in the G1, 38.8% in the G2, and 53.8% in the G3. No G1 patient developed HCC. Twenty percent and 37.2% of G2 and G3 patients, respectively, developed HCC during follow-up. A statistically significant difference was noted between the 3 groups in the occurrence of HCC (p <0.001). According to the aMAP score, 6.3% of patients had a low risk of developing HCC and 60.3% were considered to be at high risk of developing HCC. In the low-risk group, no patient developed HCC while in the high-risk group 58.8% of patients developed HCC. A statistically significant difference was noted between the two groups in the occurrence of HCC (p <0.001). The area under the ROC curve of THRI and aMAP score in the prediction of HCC development was 0.69 [95% CI: 0.61-0, 76] and 0,608 [95%CI: 0,524-0,693]), respectively.

Conclusion: The THRI score and the aMAP score seem to be useful scores in stratifying the risk of developing HCC in our population. These scores could therefore better guide screening protocols.

PO-313
Liver transplantation for fibrolamellar hepatocellular carcinoma: An analysis of the European Liver Transplant Registry

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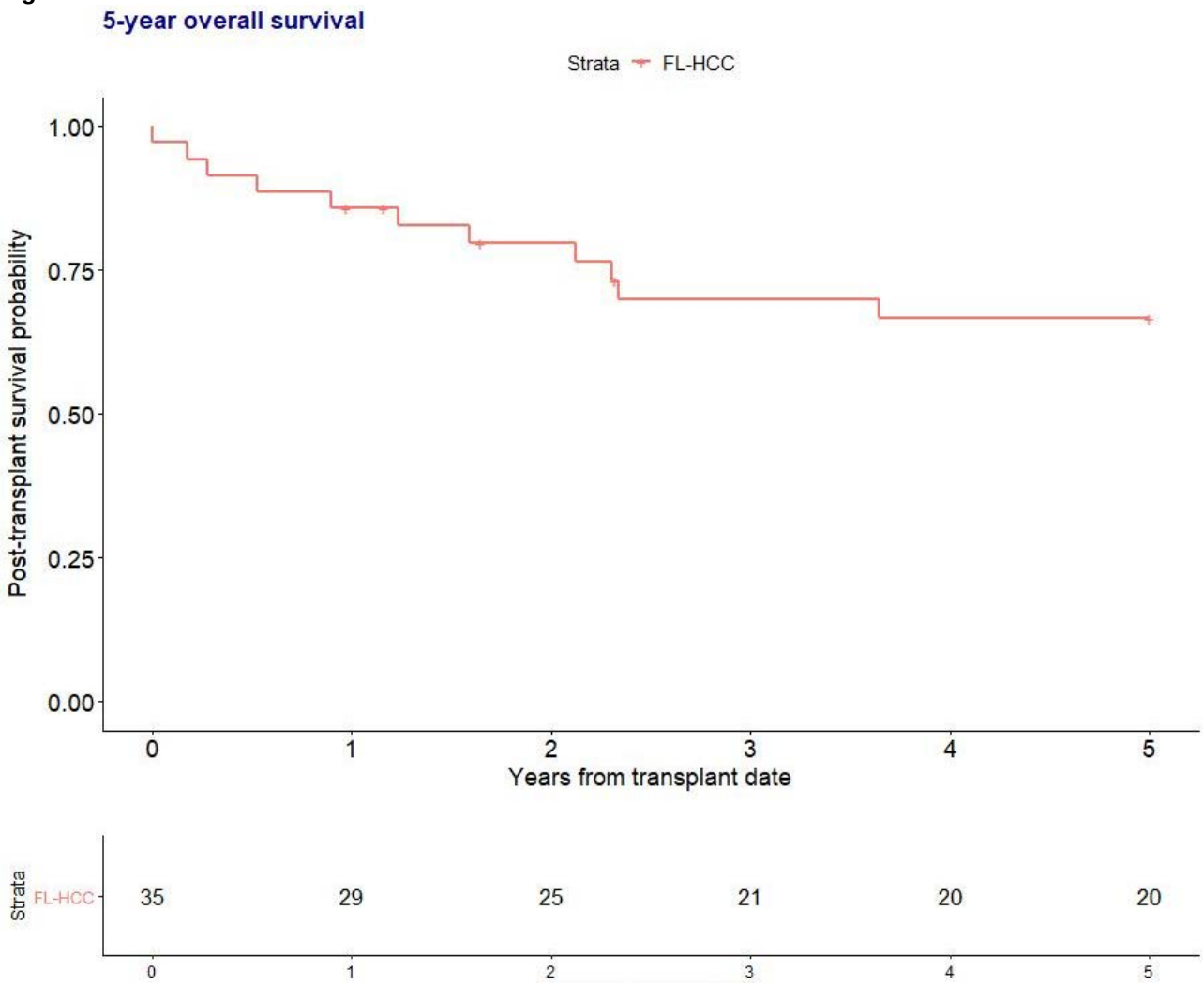
Background and aims: Due a lack of reliable data, the use of liver transplantation (LT) for fibrolamellar hepatocellular carcinoma (FL-HCC) remains under debate. We sought to increase the understanding of short- and long-term outcomes of liver transplantation for FL-HCC by analysing data from the European Liver Transplant Registry (ELTR).

Method: All centres that had registered at least one LT for FL-HCC prior to July 2021 were contacted for study participation. Only cases with a confirmed diagnosis of FL-HCC were included. Overall survival (OS) and recurrence-free survival (RFS) rates were estimated using the Kaplan-Meier method. For cumulative incidence of recurrence, death without recurrence was considered a competing event.

Results: Thirty-five FL-HCC patients from 25 different centres were included, all transplanted between 1985 and 2020. Half of them were female (51%) and the median age was 30 years (interquartile range [IQR] 23-46). At the time of listing for LT, 43% of patients had already been diagnosed with FL-HCC and were listed as such. A similar number of patients were listed for HCC (43%). The listing reason for the remaining patients was unknown (4%). Only three patients (9%) had an underlying liver disease, consisting of alcoholic liver disease (2 patients) and non-alcoholic steatohepatitis (1 patient). The median tumour number at listing was one (IQR 1-2) with a largest lesion size of 55mm (IQR 20-140). Fifteen of 35 patients (43%) received treatment before LT, consisting predominantly of liver resection (47%) and TACE (40%). Median MELD at transplant was 9 (IQR 8-12). Pre-LT tumour markers levels were: AFP median 6 (IQR 3-118), CA19.9 median 14.8 (IQR 2.7-13.0), CEA median 1.25 (0.25-2.15). The median time from listing to transplant was 51 days (IQR 9-128). At explant pathology, the median tumour number was one (IQR 1-2) with a median maximum size of the largest lesion of 60mm (32-150). Vascular invasion was present in 37% of the explanted livers. Six patients received a re-LT (17%) after their first transplant, half of them due to hepatic artery thrombosis. Recurrence occurred in 14 of the 35 patients (40%), most frequently extrahepatic (75%). At 30 days one patient (3%) had died, at 90 days two (6%). OS at 1, 3, 5-years post-LT was 86%, 70%, and 67%, whereas RFS was 77%, 62%, and 52% at similar timepoints. Cumulative incidence of recurrence was 17%, 30%, and 39% at 1, 3, and 5-years post-LT. Patients with a single tumour at explant pathology (median size 90mm, IQR 40-150) showed a 5-years survival rate of 81%, 5-years RFS of 57% and a 5-years cumulative incidence of recurrence of 43%.

Conclusion: Liver transplantation for FL-HCC yields acceptable short- and long-term survival outcomes, especially for patients with a single lesion. However, the overall recurrence rate remains high in all groups.

Figure:


5-year overall survival
Strata FL-HCC
1.00
0.75
0.50
0.25
0.00
Post-transplant survival probability
0
1
2
3
4
5
Years from transplant date
Strata
FL-HCC
35
29
25
21
20
20
0
1
2
3
4
5

PO-314

Performance of ten non-invasive liver function tests in predicting hepatocellular carcinoma development in patients with cirrhosis

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Background and aims: Hepatocellular carcinoma (HCC) is a life-threatening complication of cirrhosis requiring early and systematic screening in patients with cirrhosis.
Our objective was to evaluate the performance of ten non-invasive liver function tests in predicting HCC development in patients with cirrhosis.

Method: We performed a retrospective analysis of data from consecutive cirrhotic patients recruited from January 2010 to December 2019. In addition to the CHILD score, the following scores were calculated: MELD, albumin-bilirubin grade (ALBI), platelet-albumin-bilirubin grade (PALBI), fibrosis-index based on 4factors (FIB-4), aspartate-aminotransferase-to-platelet ratio(APRI), Lok index, cirrhosis discriminant index(CDS), King's score, Goteborg-University Cirrhosis Index(GUCI), and aspartate-aminotransferase to alanine-aminotransferase ratio(AAR).

Results: In total, 224 patients were included. The mean age was 61.02 ±13.2 and the sex-ratio was 1.6. Viral origin (54.9%) was the predominant etiologies of cirrhosis. Sixty-one patients developed HCC (27.85%). The following scores have been statistically associated with HCC development in cirrhotic patients: APRI (p=0.018); GUCI (p=0.025); ALBI (p=0.017) and MELD (p=0.033). APRI had the best area under the curve ROC (AUROC) in predicting HCC development (AUROC=0.651 [95%CI: 0.561-0.741]) followed by GUCI (AUROC=0.643 [95%CI: 0.554-0.732]). At the cut-off of 1.423, APRI had a sensibility and specificity of 70.5% and 60% respectively isn the prediction of HCC development. At the cut-off of 1.872, GUCI had a sensibility and specificity of 70.4% and 58.6% respectively in predicting HCC development in patients with cirrhosis.

Conclusion: In our study, The APRI and GUCI scores seem to be useful scores in predicting HCC development in patients with cirrhosis. These scores could therefore be included in HCC screening protocols.

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