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ABSTRACT BOOK

LIVER CANCER SUMMIT 2020

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Contents

ORAL POSTER ABSTRACT PRESENTATIONS

ePOSTER ABSTRACT PRESENTATIONS

ORAL POSTER ABSTRACT PRESENTATIONS

OP01-01YI Hepatitis B virus integration promotes large structural rearrangements and genomic instability in hepatocellular carcinomas

Camille Peneau[1 2], Sandrine Imbeaud[1 2], Tiziana La Bella[1 2], Jessica Zucman-Rossi[1 2 3]
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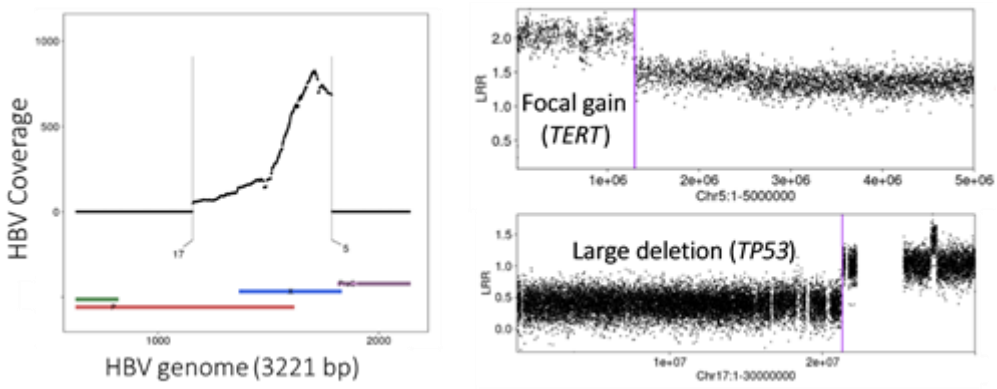
Background and aims: Hepatitis B virus (HBV) infection is the leading cause of hepatocellular carcinoma (HCC) occurrence worldwide, and not only in the setting of cirrhosis, underlying that the virus has its own oncogenic properties. HBV is a 3.2 kb enveloped DNA virus infecting hepatocytes that could integrate in human genome at the early steps of infection. Next-generation sequencing data enabled to describe HBV integration as a cancer driver alteration in large cohorts of HCC from Asian patients by identifying cancer-related genes as recurrent integration sites. However, the precise mechanism of integration and how it triggers or derives from chromosomal instability remain unclear. Our project uses a multi-omics approach in order to decipher the landscape and the chronology of HBV-related insertional mutagenesis in HCC and non-tumor liver tissues from European and African patients.
Method: The presence of HBV DNA was screened by quantitative PCR in tumor and non-tumor liver tissues from 1128 patients (97% with a European or African origin), we selected 220 HCC and their normal liver counterparts from 180 HBV-positive patients to perform viral capture. A selection of HCC samples (130 HBV-positive and 135 HBV-negative) were also sequenced with Whole Genome Sequencing (short-read or long-read) or Whole Exome Sequencing and RNA Sequencing.
Results: We identified 180 patients whose liver tissues were highly positive for HBV DNA and in most of the cases, several viral DNA forms were coexisting in the liver. 493 integration events were classified as clonal or sub-clonal, among 182 samples, and 97% of these events were observed in tumors. The only recurrent integration site observed among tumors was the *TERT* promoter region (in 46 tumors), but HBV insertions were also frequently found in centromeric and telomeric regions (respectively in 12 and 28 tumors). Moreover 74% of the HBV-integrated tumors had more than one clonal or sub-clonal insertion site. In-silico reconstruction of integrated sequences revealed the existence of frequent structural rearrangements in the viral sequence as in the human genome around the insertion breakpoints. These rearrangements were associated with copy-number variations at integration sites, including frequent deletions of tumor-suppressor genes such as *TP53* and/or amplification of oncogenes such as *TERT.*
Conclusion: This study described HBV integrated sequences and episomal DNA in HBV-related HCC from European and African populations. Our data suggest that HBV integration plays a key role in HCC progression by promoting large structural rearrangements and chromosomal instability. The characterization of these rearrangements according to the genetic and clinical features of the patients will help to define groups of HBV-infected patients with an increased risk to develop HCC.

Figure:



OP01-02YI Deleting in vivo beta-catenin degradation domain in mouse hepatocytes drives hepatocellular carcinoma or mesenchymal hepatoblastoma similarly to Apc loss-of-function

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Background and aims: One-third of hepatocellular carcinomas (HCCs) have mutations that activate the beta-catenin pathway. Most of these are gain-of-function (GOF) mutations in *CTNNB1,* encoding the beta-catenin. Surprisingly, it was found that constitutive activation of beta-catenin using deletion of its regulatory domain in exon 3 (*Ctnnb1*-ΔEx3) by Crelox strategy was not sufficient to promote hepatic tumorigenesis by itself (Harada, Cancer Res 2002). However, we developed Crelox genetic mouse model using *Apc* loss-of-functions (LOF) to generate beta-catenin activated tumours (Colnot, PNAS 2004). Considering the above and the low prevalence of *APC* mutations in human HCCs we aimed to generate hepatic tumours through *CTNNB1* exon 3 deletion (*Ctnnb1-Ex3*$^{lox/lox}$ mouse model) and to compare them to hepatic tumours generated via *Apc* LOF (*Apc*$^{Lox/Lox}$ mouse model).

Method: We used hepatic-specific and inducible *Apc*$^{Lox/Lox}$ and *Ctnnb1-Ex3*$^{lox/lox}$ mouse models as well as a hepatic-specific *in vivo* CRISPR/Cas9 approach using AAV vectors to generate hepatic tumours harbouring activation of the β-catenin pathway through GOF of *Ctnnb1* or LOF of *Apc (Apc*^{ko}*)*. Tumours generated by the Crelox and CRISPR/Cas9 models were analysed phenotypically using DNA sequencing and immunohistochemistry. Crelox-engineered tumours from each model were selected for RNA-sequencing analysis. Mouse RNAseq data were compared to RNAseq data from 72 human samples (including normal tissues, HCCs and hepatoblastomas) in an integrative analysis.

Results: Unlike what was previously reported, deletion of Ctnnb1-exon3 in mouse hepatocytes led to liver carcinogenesis by itself. Generated tumours, either *Ctnnb1*-ΔEx3 or *Apc*^{ko}, are indistinguishable depending on the mutation. Both *Ctnnb1-Ex3*$^{lox/lox}$ and *Apc*$^{Lox/Lox}$ mouse models induced two phenotypically different tumours, either differentiated or undifferentiated. Integrative analysis of human and mouse tumours showed that mouse differentiated tumours are close to well differentiated, beta-catenin activated, HCCs while undifferentiated ones are closer to human mesenchymal hepatoblastomas, also characterized by their activation of the beta-catenin signalling.

Conclusion: Contrary to popular belief, constitutive activation of beta-catenin through deletion of its regulatory domain in adult mouse liver drives HCC or hepatoblastoma similarly to *Apc* LOF. Moreover, CRISPR approach are now easily available for rapid combination with existing mouse models.

Figure:

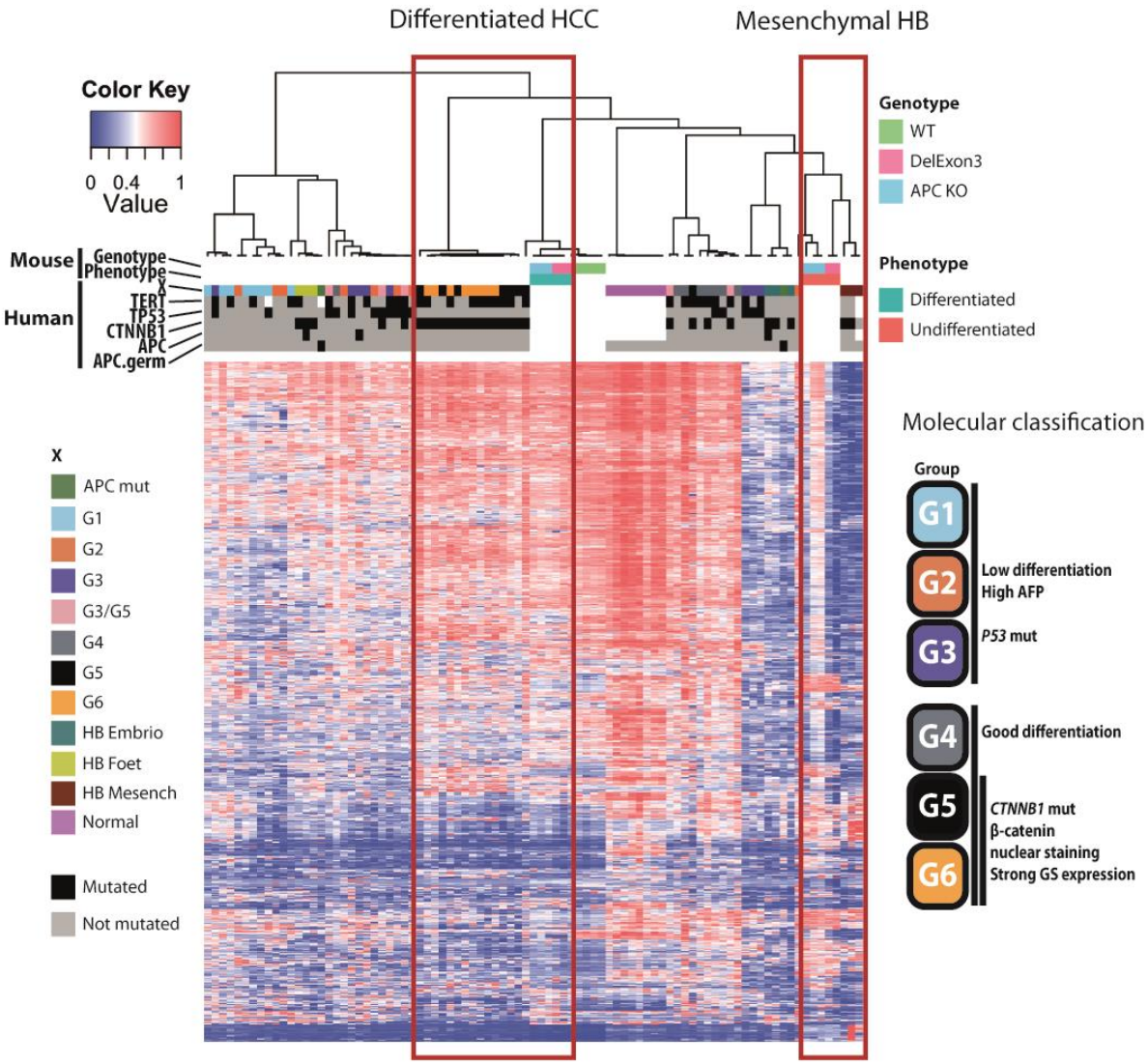

Figure : Consensus clustering after integrative analysis of mouse and human liver tumors. Mouse liver tumors from both phenotype generated with *Apc*$^{ko\text{-}lox}$ and *ßcat-ex3*$^{ko\text{-}lox}$ models were compared to a human cohorte of HCC (representing tumors from Zucman-Rossi's lab molecular classification; Calderaro et al, 2017) and hepatoblastoma (HB). This analysis showed that mouse differentiated tumors are closer to human well differentiated G5-G6 HCC while mouse undifferentiated tumors are closer to human mesenchymal hepatoblastomas.

OP02-01 FIGHT-202: a phase 2 study of pemigatinib in patients (pts) with previously treated locally advanced or metastatic cholangiocarcinoma (CCA)

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Background and aims: Fibroblast growth factor receptor (FGFR) 2 alterations are implicated in cholangiocarcinoma (CCA). Pemigatinib is a selective, potent, oral FGFR1, 2, and 3 inhibitor. We present data from a phase 2, open label, single arm study of pemigatinib in patients with previously treated locally advanced or metastatic CCA (NCT02924376).

Method: Eligible adults had disease progression after at least 1 prior treatment and documented FGF/FGFR gene status. Patients assigned to cohorts A (FGFR2 gene rearrangements/fusions), B (other FGF/FGFR gene alterations), or C (no FGF/FGFR gene alterations) received oral pemigatinib 13.5 mg QD (21-day cycle; 2 weeks on, 1 week off) until disease progression/unacceptable toxicity. Primary endpoint was centrally confirmed objective response rate (ORR; cohort A); secondary endpoints were ORR (cohorts B, A + B, and C); duration of response (DOR), disease control rate (DCR), progression-free survival (PFS), overall survival (OS), and safety.

Results: At data cutoff (Mar 22, 2019), 146 patients were enrolled (cohort A, n = 107; B, n = 20; C, n = 18; 1 patient undetermined). Median (range) age was 59 (26–78) years; 61% and 39% had 1 and at least 2 prior therapies, respectively. Fewer patients discontinued therapy in cohort A (71%) vs B and C (each 100%), mainly for progressive disease (53%, 75%, and 67%, respectively). ORR in cohort A was 35.5% (95% CI, 26.5%–45.4%), with 3 complete responses; median DOR was 7.5 (95% CI, 5.7–14.5) months, DCR was 82% (95% CI, 74%–89%), median PFS and median OS were 6.9 (95% CI, 6.2–9.6) and 21.1 (14.8–not reached) months (OS not mature at cutoff). In cohorts B and C, no patient achieved a response. Overall, most common adverse events (AEs) were hyperphosphatemia (60%; grade 3–4, 0%), alopecia (49%; 0%), diarrhea (47%; 3%), fatigue (42%; 5%), nail toxicities (42%; 2%), and dysgeusia (40%; 0%). Hyperphosphatemia was managed with diet modifications, phosphate binders, if needed; diuretics or dose reductions/interruptions. Discontinuation, dose reduction and interruption due to AEs occurred in 9%, 14% and 42% of patients, respectively.

Conclusion: These data support pemigatinib as a potential treatment option for previously treated patients with CCA harboring FGFR2 gene rearrangements/fusions.

OP02-02 An imaging genomics approach identifies molecular underpinnings and actionable targets for hepatocellular carcinoma with fluorodeoxyglucose uptake in positron emission tomography scans

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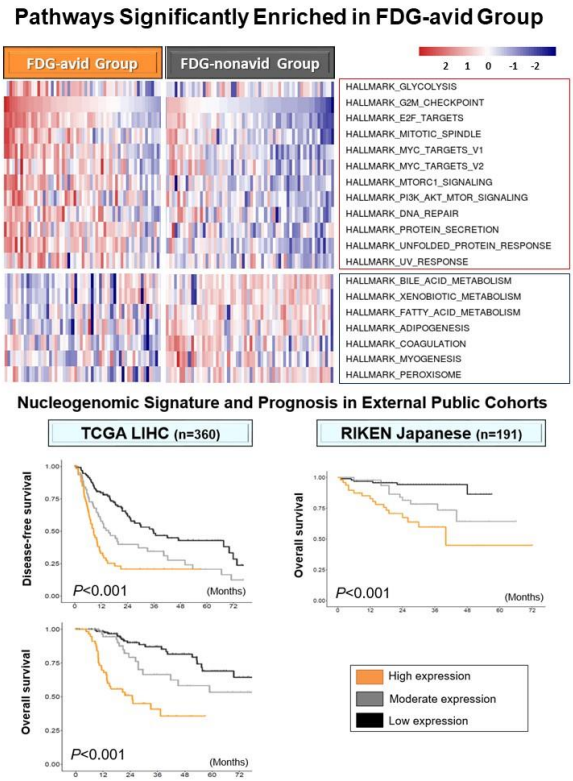
Background and aims: Although [18F]fluorodeoxyglucose (FDG) uptake during positron emission tomography (PET) predicts poor outcome in patients with hepatocellular carcinoma (HCC), biologic basis and potential targetability for the FDG-uptake lesions is not fully understood.

Method: We used mRNA sequencing, whole exome sequencing, and copy number variation data obtained from 117 in-house HCC patients who underwent FDG-PET/CT followed by tumor resection to explore genomic and molecular surrogates for tumors with hypermetabolic uptake. Based on the visual grading system, all patients were divided into the two groups: FDG-avid (n=57, 48.7%) and FDG-nonavid groups (n=60, 51.3%).

Results: The FDG-avid group had larger tumors, and higher prevalence of vascular invasion and elevated serum AFP (≥200 ng/mL) than the FDG-nonavid set (ps<0.05). Patients with hypermetabolic HCC showed significantly worse disease-free survival after resection than the counterparts (p<0.05). Single sample gene set enrichment analysis (ssGSEA) revealed functional differences between the two groups of subjects: glycolysis, cell-cycle related and MTORC signaling pathways were enriched in the FDG-avid set. The higher frequencies of somatic mutations in genes related to the corresponding pathways were consistently observed in the FDG-avid group. Positive FDG uptake was also significantly associated with higher levels of recurrent copy number changes. When we developed and validated a 'nucleogenomic signature' that consisted of the top 100 genes upregulated in hypermetabolic HCCs, a subset of patients with the curated gene set showed worse survival outcomes in the two public datasets (TCGA and RIKEN).

Conclusion: Our high-throughput radiogenomic analysis indicates that both mTOR and cell-cycle signaling pathways are more likely altered in the highly relapsable HCCs with FDG accumulates. The metabolic phenotype of HCC can serve as an imaging surrogate for risk stratification and tailored treatment in the diseased patients.

Figure:


Pathways Significantly Enriched in FDG-avid Group
FDG-avid Group
FDG-nonavid Group
2 1 0 -1 -2
HALLMARK_GLYCOLYSIS
HALLMARK_G2M_CHECKPOINT
HALLMARK_E2F_TARGETS
HALLMARK_MITOTIC_SPINDLE
HALLMARK_MYC_TARGETS_V1
HALLMARK_MYC_TARGETS_V2
HALLMARK_MTORC1_SIGNALING
HALLMARK_PI3K_AKT_MTOR_SIGNALING
HALLMARK_DNA_REPAIR
HALLMARK_PROTEIN_SECRETION
HALLMARK_UNFOLDED_PROTEIN_RESPONSE
HALLMARK_UV_RESPONSE
HALLMARK_BILE_ACID_METABOLISM
HALLMARK_XENOBIOTIC_METABOLISM
HALLMARK_FATTY_ACID_METABOLISM
HALLMARK_ADIPOGENESIS
HALLMARK_COAGULATION
HALLMARK_MYOGENESIS
HALLMARK_PEROXISOME
Nucleogenomic Signature and Prognosis in External Public Cohorts
TCGA LIHC (n=360)
RIKEN Japanese (n=191)
Disease-free survival
Overall survival
P<0.001
(Months)
High expression
Moderate expression
Low expression

OP02-03 Efficacy and safety of atezolizumab + bevacizumab vs sorafenib in Chinese patients with unresectable HCC in the phase III IMbrave150 study

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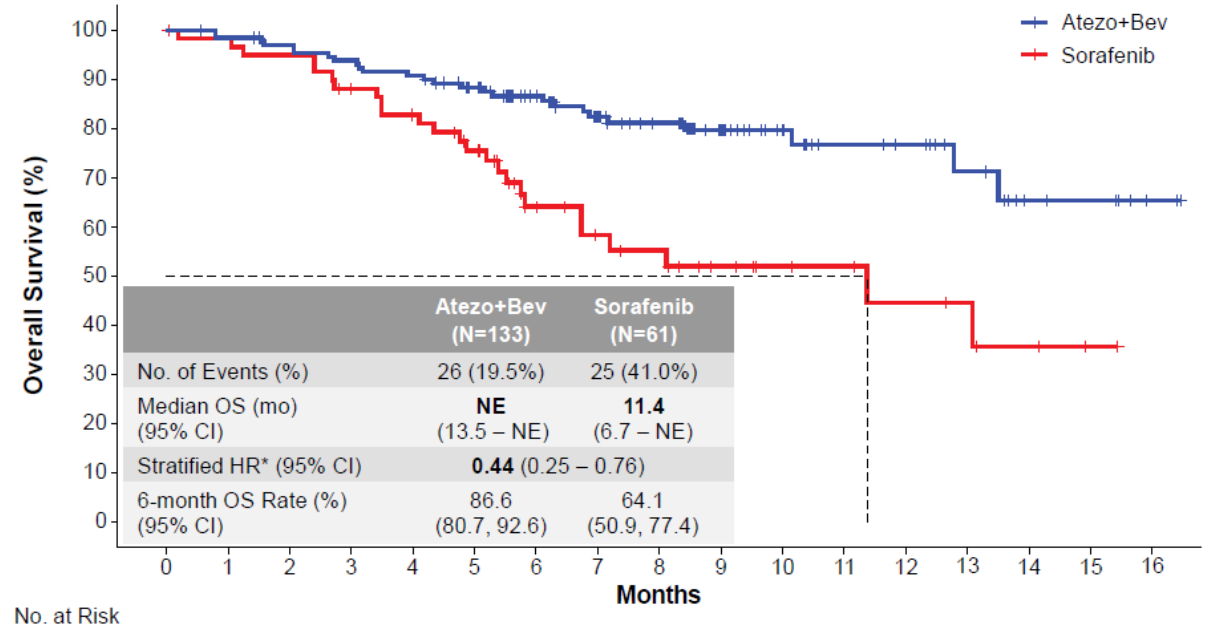
Background and aims: In the global IMbrave150 study, atezolizumab (atezo) + bevacizumab (bev) demonstrated a significant improvement vs sorafenib (sor) for OS and independent review facility (IRF)-assessed PFS per RECIST 1.1 in patients (pts) with unresectable HCC who had not received prior systemic therapy (tx) (Cheng ESMO Asia 2019). We report results in Chinese pts from the IMbrave150 global and China extension cohorts.

Method: Systemic tx-naïve pts with unresectable HCC were randomized 2:1 to receive atezo 1200mg IV q3w + bev 15mg/kg IV q3w or sor 400mg bid until unacceptable toxicity or loss of clinical benefit. Co-primary endpoints were OS and IRF-assessed PFS per RECIST 1.1. Key secondary endpoints included IRF-objective response rates (ORR) per RECIST 1.1 and IRF-ORR per HCC modified (m)RECIST.

Results: Of 194 Chinese pts (137, global; 57, extension), 133 were randomized to atezo+bev and 61 to sor tx. Baseline demographics were balanced between tx arms. Chinese pts had higher rates of HBV, BCLC Stage C, macrovascular invasion and/or extrahepatic spread, and α-fetoprotein ≥400 ng/mL vs global pts. At a median follow-up of 7.2 mo for atezo+bev and 5.6 mo for sor, the OS stratified HR was 0.44 (95% CI 0.25–0.76) (Figure). The PFS stratified HR was 0.60 (95% CI 0.40–0.90); the median PFS was 5.7 mo with atezo+bev and 3.2 mo with sor. ORR was 25% vs 7% per IRF RECIST 1.1 and 30% vs 9% per IRF HCC mRECIST for atezo+bev vs sor, respectively. Atezo+bev delayed time to deterioration of pt-reported quality of life vs sor. Median tx duration was 6.0 mo for atezo, 5.5 mo for bev and 2.8 mo for sor. Gr 3/4 AEs occurred in 59% of 132 atezo+bev-treated and 47% of 58 sor-treated pts; gr 5 AEs occurred in 2% and 3%, respectively. AEs led to withdrawal from all tx components in 2% of both arms.

Conclusion: In Chinese pts with unresectable HCC who had not had prior systemic tx, clinically meaningful improvements in OS and PFS were seen with atezo+bev vs sor despite generally increased negative prognostic factors vs the global pts. Overall, these data were consistent with the global results. Atezo+bev was generally well-tolerated with manageable toxicities and the safety profile was consistent with the known risks of the individual study tx and underlying disease. Atezo+bev may be a practice-changing tx for Chinese HCC pts.

Figure:



	Atezo+Bev (N=133)	Sorafenib (N=61)
No. of Events (%)	26 (19.5%)	25 (41.0%)
Median OS (mo) (95% CI)	**NE** (13.5 – NE)	**11.4** (6.7 – NE)
Stratified HR* (95% CI)	**0.44** (0.25 – 0.76)	
6-month OS Rate (%) (95% CI)	86.6 (80.7, 92.6)	64.1 (50.9, 77.4)

No. at Risk

Atezo+Bev	133	130	125	121	117	106	86	70	60	45	28	21	19	13	7	6	2
Sorafenib	61	58	56	50	46	39	25	19	17	12	9	8	6	5	3	1	0

*Unstratified HR is 0.38 (95% CI: 0.22–0.66). Stratification factors include MVI and/or EHS (presence vs. absence) and AFP level (<400 ng/mL vs. ≥ 400 ng/mL) at screening per IxRS

Clinical Cut-off Date: 29 Aug 2019

ePOSTER ABSTRACT PRESENTATIONS

P01-01YI Clinical strategy of diagnosing patients with hepatocellular carcinoma based on latent transforming growth factor-beta binding protein 1

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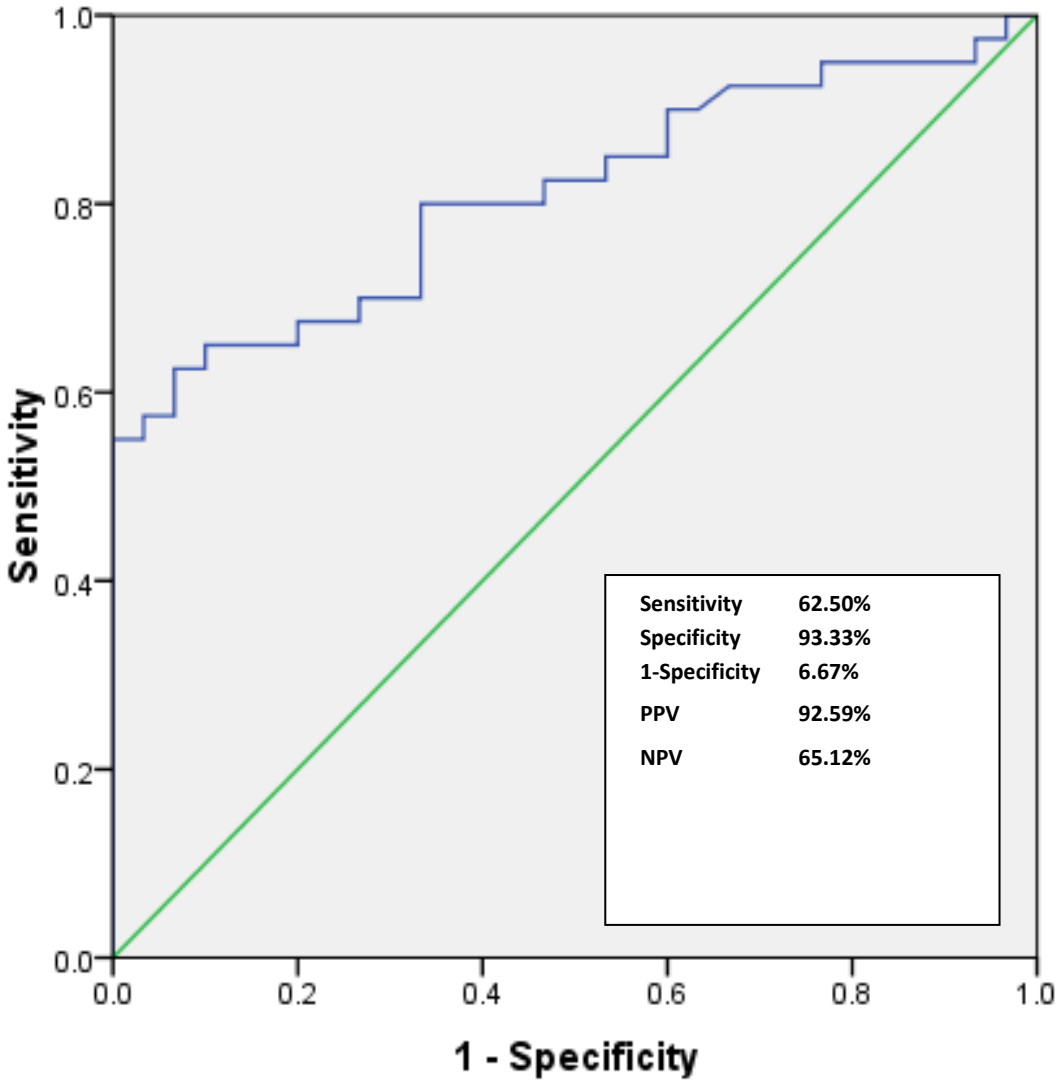
Background and aims: Hepatocellular carcinoma (HCC) is the leading cause of cancer related death worldwide. The latent transforming growth factor-beta binding protein 1 (LTBP-1) is a secreted protein and considers as a part of the extracellular matrix (ECM). LTBP-1 targets transforming growth factor-beta 1 (TGF- β 1) and localizes it to ECM by interacting with integrin and fibronectin. Previous studies showed that the immunohistochemistry staining of LTBP-1 was extremely strong in the tumor stroma of malignant mesothelioma, pancreatic ductal adenocarcinoma and ovarian carcinoma. The current study aimed to evaluate the diagnostic role of LTBP-1 as a biomarker to distinguish HCC from patients with liver cirrhosis.

Method: The current study was conducted as a cross-sectional and case-control based study. It was approved by the Institutional Review Board National Liver Institute (IRB number 00003413). The current study included 90 individuals; 40 HCC patients (11 female and 29 males with the mean age of 53 years), 30 patients with cirrhosis (7 female and 23 males with the mean age of 54 years), and 20 healthy volunteers as a control group (1 female and 19 males with the mean age of 49 years). The serum level of LTBP-1 was measured by enzyme-linked immunosorbent assay (ELISA).

Results: The LTBP-1 level was significantly higher in HCC patients than healthy and patients with cirrhosis. Furthermore, there was a significant ($p<0.001$) association between the level of LTBP-1 and CLIP and BCLC in HCC patients. Moreover, LTBP-1 levels were significantly ($p=0.01$) associated to child pugh grade in patients with cirrhosis and HCC. ROC curve analyses revealed that LTBP-1 showed a better diagnostic performance (AUC=0.970, Sensitivity: 82.50%, Specificity: 96.67%, PPV: 97.06%, NPV: 80.56%) in distinguishing HCC from cirrhosis patients, compared to AFP (AUC=0.810, Sensitivity: 62.50%, Specificity: 93.33%, PPV: 92.59%, NPV: 65.12%). the level of LTBP-1 in HCC patients was significantly ($p<0.001$) associated with CLIP score. AFP was not significantly ($p=0.098$) associated with CLIP score. Concerning to BCLC, There was a significant ($p<0.001$) association between the serum level of LTBP- 1 and BCLC score in HCC patients. The LTBP-1 level was gradually increased with the progress in BCLC score, where, the level was 46.8 in score 4 against 26.6 in score 0.

Conclusion: The serum levels of LTBP-1 exhibited gradually increased trend in healthy individuals, liver cirrhosis and HCC patients. Serum LTBP-1 might be a potential serum marker to discriminate HCC from liver cirrhosis patients due to its high sensitivity and specificity, compared to AFP. LTBP-1 might be a promising diagnostic biomarker for HCC although; we recommended that future studies on large number of patients are required to validate these results.

Figure:


Sensitivity
1 - Specificity
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
Sensitivity 62.50%
Specificity 93.33%
1-Specificity 6.67%
PPV 92.59%
NPV 65.12%

P01-02 Radiological and pathological characteristics associated with aggressive intrasegmental recurrence of hepatocellular carcinoma after radiofrequency ablation

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Background and aims: Aggressive intrasegmental recurrence (AIR) is a severe form of local recurrence occurring after a successful HCC treatment by radiofrequency ablation (RFA). It is defined by the simultaneous development of multiple recurrent nodules (at least 3) or by a diffuse infiltrative mass accompanied by a tumor thrombus, in the treated segment. We aimed to identify in this retrospective study radiological and/or histological characteristics predictive for this recurrence.
Method: We retrospectively included all patients referred to our institution for a first treatment of HCC by RFA from january 2007 to december 2017, and selected among them patients with both initial HCC tumor biopsy and standard triphasic cross-sectional imaging available. Baseline clinical and biological features were recorded, all pretherapeutic imaging studies were reviewed and the following parameters were assessed: size, number of nodule, tumor margins, peri-vascular location, tumor capsule, peritumoral arterioportal shunt, atypical enhancement characteristics. Biopsy samples have been reassessed for histological subtyping, Edmondson score, and immunohistochemical expression of biliary markers (EpCAM and/or CK19) and endothelial-specific-molecule-1 (ESM-1). Follow-up imaging was reviewed and the first recurrence was considered as the primary endpoint, classified as non-aggressive local recurrence, aggressive intra-segmental recurrence or intrahepatic distant recurrence.
Results: Among 611 naïve patients treated by RFA, 212 were included (168 men; mean: 67.1 yrs [40-87]; 181 cirrhosis, 142 BCLC0/A). The mean tumor size was 28.6 mm [9-90]. Mean follow-up was 28 months [0-116]. AIR occurred in 21/212 patients (10%) within a median delay of 14 months [1-43] after successful ablation. Twelve of 21 (57%) patients with AIR and 58 out of 191 (30%) patients without AIR died during follow up died during follow-up (log-rank test, p=0.0001). In univariate analysis, characteristics associated with AIR were: non smooth tumor margin (HR:4.8 [2.03 ;11.31], p=0.0003), histological massive macro-trabecular subtype (HR:6.14 [2.53 ;14.88], p=0.00005) and ESM-1 expression (HR:3.11 [1.28 ;7.54], p=0.01). Multivariate analysis showed that non smooth tumor margins (HR:3.7 [1.57 ;9.06], p=0.002) observed in 45/212 patients (21%) and macro-trabecular massive histological subtype (HR:3.8 [2,47 ;10], p=0.005) observed in 25/212 patients (12%) were independently related to a higher risk of AIR occurrence.
Conclusion: AIR was associated with 2 baseline radiological and histological features: non smooth borders and macro-trabecular massive subtype. Given the strong impact of AIR on survival, we suggest that these features would be useful to stratify therapeutic strategy.

P01-03YI Hepatocellular carcinoma: Most common presentation and its association with portal vein thrombosis in different age groups

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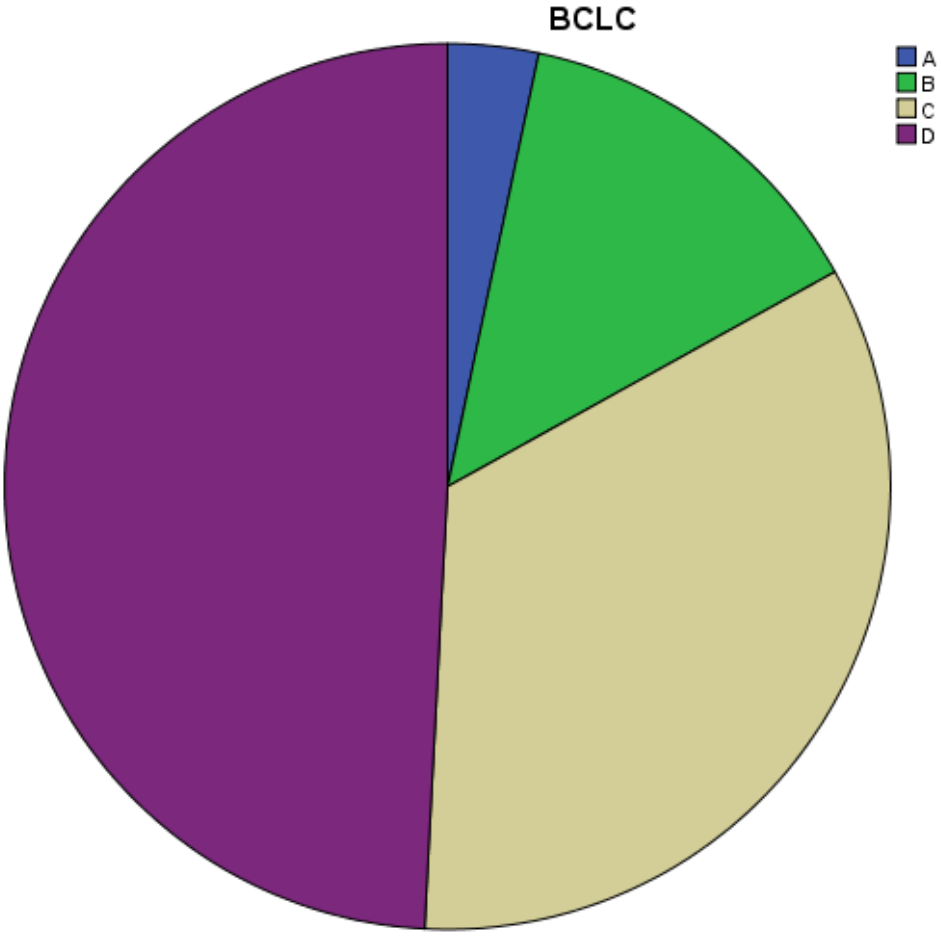
Background and aims: Hepatocellular Carcinoma (HCC) is the most serious complication in the cirrhotic. HCC is believed to be the 2nd cause of cancer deaths around the globe even in the presence of different treatment modalities. We aimed to determine the most common presentation of HCC and the presence of portal vein thrombosis in different age groups among our Pakistani population

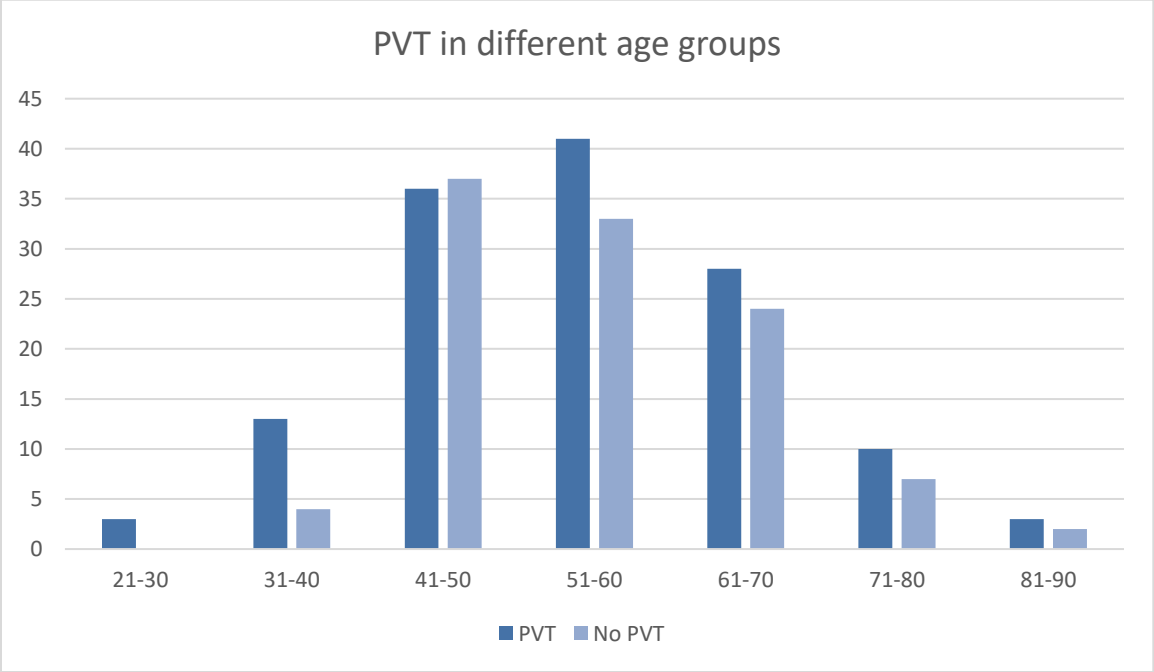
Method: A cross-sectional study was conducted in the Asian Institute of Medical Sciences, Hyderabad, Pakistan. Data was collected after the consensual agreement of 242 patients. Diagnosis of HCC was based on CT scan or ultrasound combined with Alpha-fetoprotein (AFP) level. Parameters included were age, Barcelona Clinic of Liver Cancer (BCLC) class, Child-Pugh Class (CTP), AFP, absence or presence of Portal Vein Thrombosis (PVT) and others.

Results: A total of 242 patients with mean age of 55 years included, 198 (81%) were male. The most common aetiology remained same as HCV in 170 (70%), followed by HBV 28 (11.5%) and Alcohol 18 (7.4%). Most of the patients presented late with BCLC-D (49.2%), followed by BCLC-C and BCLC-B (33.4% and 13.4% respectively), while BCLC-A was only 3.3%. CTP-B was the most common class presented in 108 (43.7%) followed by CTP-C (35.1%) and CTP-A (20.2%).
PVT was found in total 134 (54.3%) patients, it was present in 16 out of 20 patients(80%) of age between 21 to 40, while 36 out of 73 patients (49.3%) of 5th decade, and 3 out of 5 (60%) in patients of more than 80 years of age.

Conclusion: HCV Cirrhosis is the main cause of HCC in our region. PVT was significantly common and observed in younger population presented with HCC. A Large number of population with CLD presented late with advanced disease and the major reason was the lake of knowledge and awareness. Delay in the diagnosis is the main root of making HCC incurable. There is a crucial need to spread knowledge and create medical awareness to diagnose HCC at an early stage.

Figure:


BCLC
A
B
C
D


PVT in different age groups
45
40
35
30
25
20
15
10
5
0
21-30
31-40
41-50
51-60
61-70
71-80
81-90
PVT
No PVT

P01-04 Randomized, open label, perioperative phase 2 study evaluating nivolumab alone or nivolumab plus ipilimumab in patients with resectableHCC

Ahmed Kaseb[1], hop sanderson tran cao[2], aliya qayyum[3], dan duda[4], Luis Vence[5], jorge blando[5], shalini singh[5], sunyoung lee[1], Kanwal Raghav[1], divya sakamuri[1], li xu[1], kristen carter[1], tan dongfeng[6], Asif Rashid[6], jean vauthey[2], ching wei david tzeng[2], Yehia Abugabal[1], thomas aloia[7], yun shin chun[2], james yao[1], robert wolff[1], hesham amin[6], james allison[5], Padmanee Sharma[8]
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Email: akaseb@mdanderson.org

Background and aims: In HCC, surgical resection is associated with high recurrence rates, and no effective neoadjuvant or adjuvant therapies currently exist. Immunotherapy using anti-PD-1 antibodies has shown promised but limited increase in survival in advanced disease. To maximize the benefit, we are studying the efficacy and safety of anti–PD-1 (nivolumab) and anti–CTLA-4 (ipilimumab) antibodies against HCC for resectable HCC.

Method: This is a randomized phase II trial of nivolumab (Arm A) or nivolumab + ipilimumab (Arm B) as pre-operative treatment for patients (pts) with HCC who are eligible for surgical resection. Pts are given nivolumab 240 mg every 2 weeks (wks) for a total of 6 wks. Pt in Arm B are treated concurrently with ipilimumab 1 mg/kg every 6 wks. Surgical resection occurs within 4 wks after last cycle of therapy. Pts continue adjuvant immunotherapy for up to 2 years after resection. The primary objective is the safety/tolerability of nivolumab +/- ipilimumab. Secondary objectives include overall response rate, complete response rate and time to progression. Exploratory objectives include evaluating the pre- and post-treatment immunological changes in tumor tissues and peripheral blood.
Results: Twenty-six patients were enrolled at the time of this interim analysis, of which 20 have evaluable data.Most pts (55%) were between 60-70yo and male (75%).Seven pts were HCV-positive, 7 had HBV and 6 had no hepatitis. 20 patients proceeded with resection as planned but surgery was aborted for 3 patients (1 for frozen abdomen and 2 development of contralateral liver nodule). Three are still receiving preoperative therapy. Pathologic complete response (pCR) was observed in 5/20 evaluable patients – 2 in Arm A and 3 Arm B (25% pCR rate). Five patients in Arm B and 1 in Arm A experienced grade 3 or higher toxicity prior to surgery. No grade 4 or higher toxicity were observed.
Conclusion: We report a pCR rate of 25% for resectable HCC after preoperative immunotherapy in a randomized phase II pilot trial. Treatment was safe and surgical resection was not delayed. The study is ongoing. These promising results may contribute to a paradigm shift in the perioperative treatment of resectable HCC.

Figure:

Figure 1. Trial Design

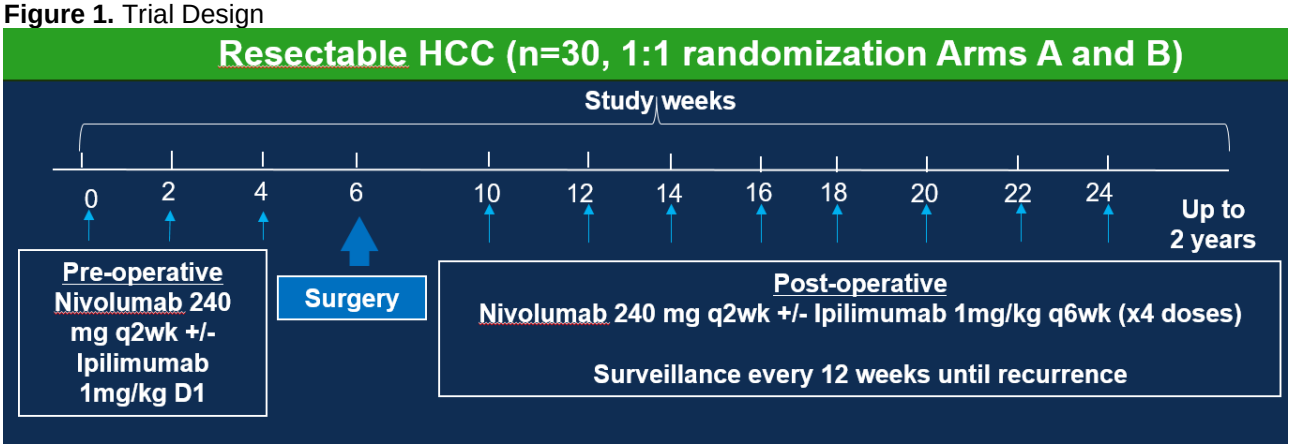


Figure 2. Pre-and post-treatment analysis of tumor-infiltrating cells in the tumor microenvironment

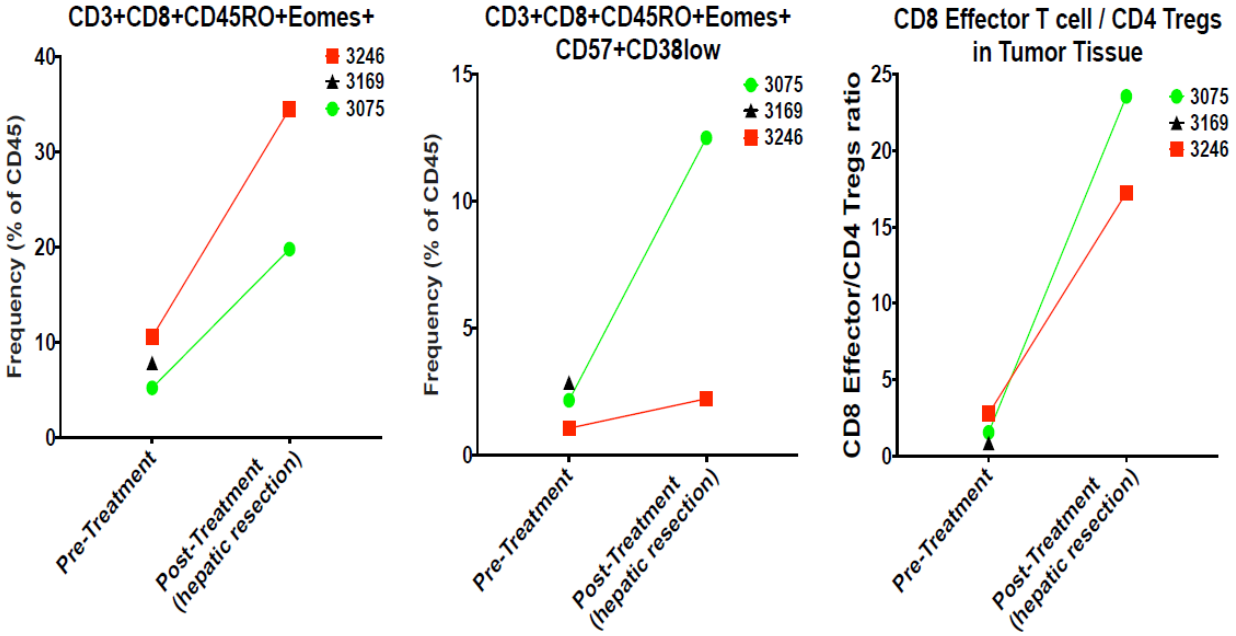
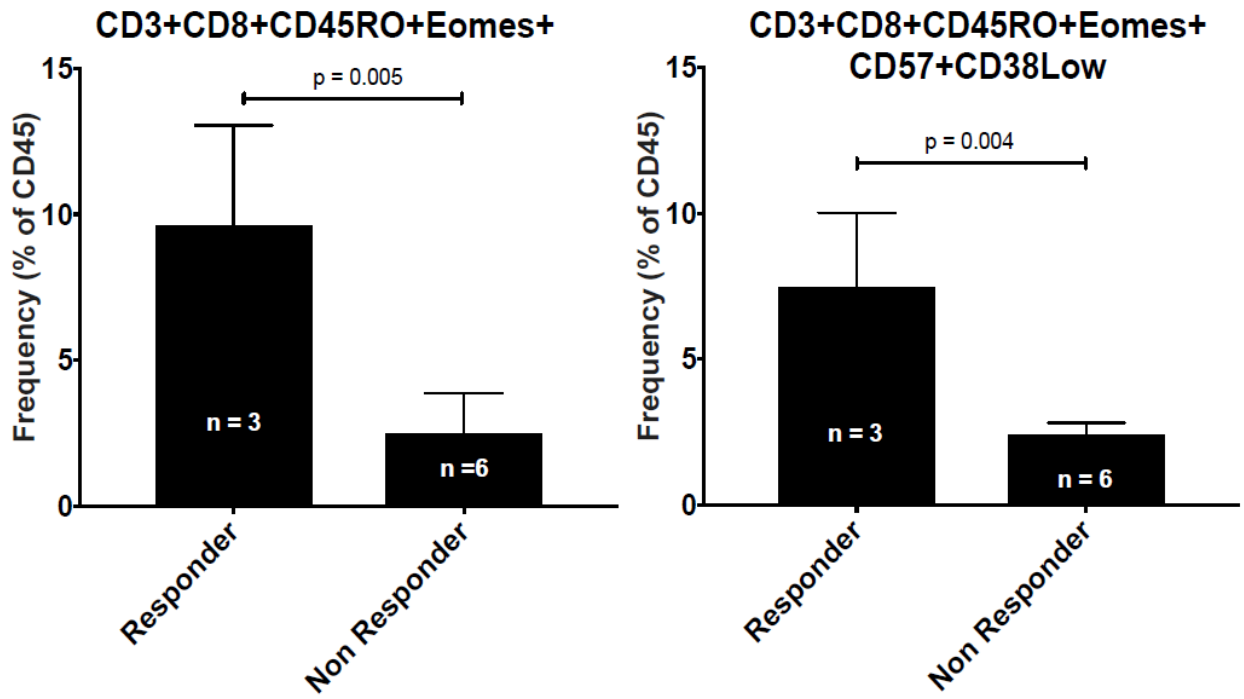


Figure 3. Comparison between the frequency of Teff clusters in the Pre-treatment samples from responders compared to the cluster frequency of Pre-treatment samples from non-responders



P01-05YI Prognostication of hepatocellular carcinoma under Sorafenib: External validation of the PROSASH-II model

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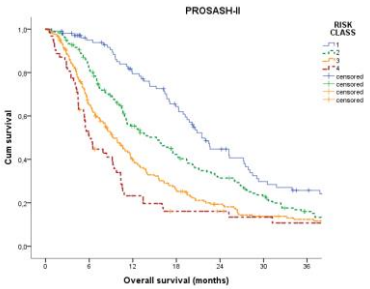
Background and aims: Prognostic classifications for patients treated with sorafenib for hepatocellular carcinoma (HCC) can be an useful tool to facilitate stratification in trials and inform clinical decision making. Very recently, Labeur and colleagues developed the PROSASH-II model, which performed better than other existing models in predicting the overall survival (OS) of sorafenib-treated patients. As this study included a 4-center training set and a single-center validation, further validation in multicenter cohorts are needed to understand the full potential of the PROSASH-II model. Aim of our study was to verify the stratification performance of the PROSASH-II and comparing with other existing prognostic models.

Method: We analyzed a large retrospective-prospective database gathering the clinical data of 552 patients from 7 Italian centres, who were prescribed with sorafenib between 2008 (date of licence in Italy) and 2017. The PROSASH –II score was calculated as proposed by its creators [(-0.0337 x albumin in g/l) + (0.315 x Ln(bilirubin in µmol/l) + (0.295 x macrovascular invasion, where 0=No and 1=Yes) + (0.181 x extrahepatic spread, where 0=No and 1=Yes) + (0.0336 x Largest tumour size in cm) + (0.0703 x Ln(AFP U/L)). It was subsequently categorized as follows: ≤-0.0760 (risk group 1), >-0.0760 to ≤0.355 (risk group 2), >0.355 to ≤0.858 (risk group 3) and >0.858 (risk group 4). The performance of the PROSASH-II was compared with those of BCLC, ALBI, HAP score and SAP score.

Results: The PROSASH-II stratification significantly discriminated patients, with a median OS of 21.5 months for risk group 1, 15.3 months for risk group 2, 9.3 months for risk group 3, and 5.9 months for risk group 4.Using risk group 1 as a reference, the hazard ratio was 1.52, 2.04, and 3.0 for risk groups 2,3, and 4, respectively. PROSASH-II showed improved discrimination (C-index 0.61) compared with existing prognostic scores (C-index≤0.57)

Conclusion: Our results validate the PROSASH-II as an effective prognostic classification model in a large Italian population of sorafenib-treated patients. We also confirm a slightly better performance of the PROSASH-II compared with the HAP and SAP scores.

Figure:


PROSASH-II
RISK CLASS
1
2
3
4
censored
censored
censored
censored
Cum survival
1,0
0,8
0,6
0,4
0,2
0,0
0
6
12
18
24
30
36
Overall survival (months)

P01-06YI Natural history of hepatobiliary hypointense-only found at magnetic resonance imaging with Gd-EOB-DTPA in cirrhotic patients: Time-dependent effects of dimensional increase and diffusion weighted imaging alterations

Luca Ielasi[1], Matteo Renzulli[2], Francesco Tovoli[1], Irene Pettinari[2], Francesca Benevento[1], Alessandro Granito[1]
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Email: francesco.tovoli2@unibo.it

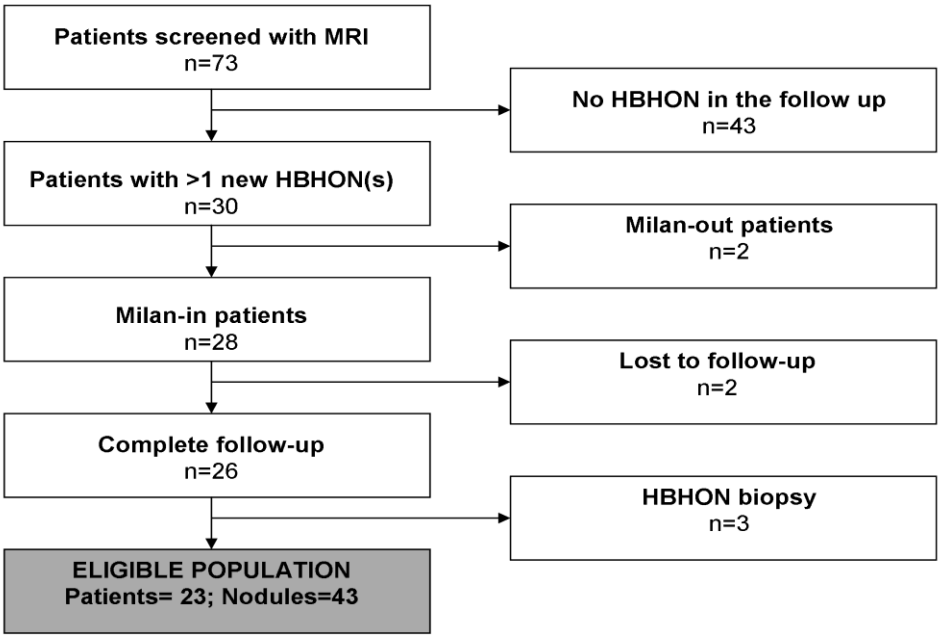
Background and aims: HB hypointense-only nodules (HBHONs) are frequently found in cirrhotic patients performing magnetic resonance imaging. Some HBHONs can transform into overt hepatocellular carcinoma (HCC) overtime. Studies including only de novo HBHON (i.e. which were absent at previous MRI examinations) and using rigorous time-dependent analyses are still lacking

Method: We evaluated consecutive patients performing MRI in the surveillance programs of HC due to a poor ultrasound visualization of the liver, analyzing only HBHONs appearing during the program (availability of a previous MRI performed no more than 6 months before the appearance of HBHONs to confirm that the nodule was not previously present). To better define the clinical repercussion of the detection of HBHONs, we excluded patients for which this finding would not have altered the clinical decision-making process (i.e patients with a concurrent Milan-out HCC at the time of the HBHON detection). The time-to-transformation (TTT) to HCC was correlated with the baseline characteristics of HBHON and with time-dependent modifications during the follow-up.

Results: Twenty-three patients and 43 nodules were included. After a median follow-up of 31.7 months, 19 nodules (45.2%) transformed into HCC. The 1-year and 2-year transformation rates were 11.9 and 31.0%, respectively. The median TTT was 17.8 months (95% CI 15.4-20.2). At the multivariable Cox regression with time-dependent covariates, the risk of evolution was 4-time increased after a dimensional increase >2mm for the baseline and 5-time increased after the appearance of diffusion-weighted imaging (DWI) alterations. The median TTT shortened to 12.1 months (range 3.1-12.1) following the appearance of DWI alterations and to 4.9 months (range 3.1-34.3) after the first dimensional increase. Diameter of the nodule at its first appearance, previous/concurrent HCC, baseline T1 and T2 parameters were no related to an increased risk of transformation.

Conclusion: Examining only de novo HBHON, we provided for the first time relevant information about the natural history of these nodules. If confirmed in collaborative studies with larger populations, our data would pave the way for important policy-making decisions about the follow-up of HBHONs.

Figure:


Patients screened with MRI
n=73
No HBHON in the follow up
n=43
Patients with >1 new HBHON(s)
n=30
Milan-out patients
n=2
Milan-in patients
n=28
Lost to follow-up
n=2
Complete follow-up
n=26
HBHON biopsy
n=3
ELIGIBLE POPULATION
Patients= 23; Nodules=43

P01-07 The concept of time-varying therapeutic hierarchy for patients with hepatocellular carcinoma: A multicenter cohort study. On behalf of the ITA.LI.CA. study group

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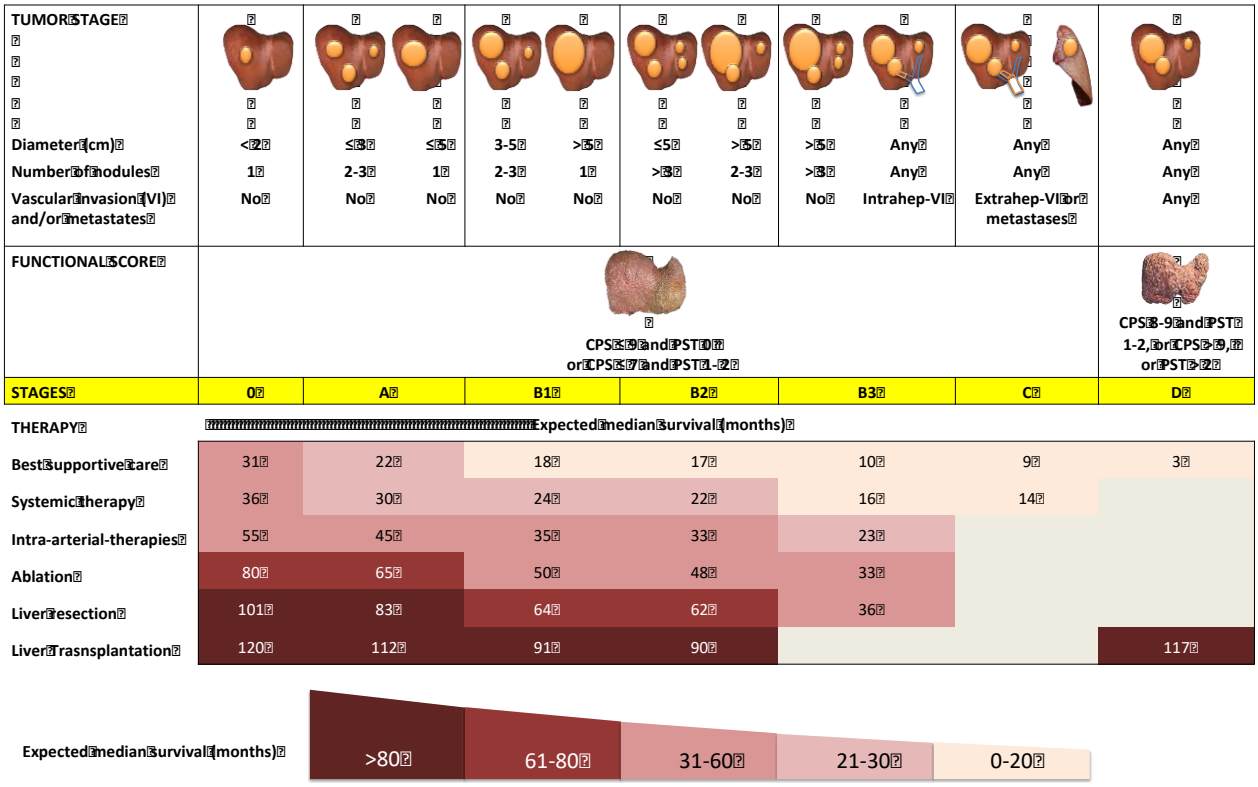
Background and aims: While prognostic studies usually focus only on the first (or the main) treatment, the oncological history of patients with hepatocellular carcinoma (HCC) is characterized by a sequence of different treatment approaches. We sought to evaluate the survival benefit of sequential HCC therapies using a time varying multivariable survival model applied to a large cohort of HCC patients.

Method: We considered 6191 treatment strategies performed in 2800 HCC patients recorded in a prospectively collected multicenter Italian database from 2008 trough 2016.
We carried out a time-varying (TV) survival analysis where the total follow-up time for each patient was split into several observations accounting for each treatment procedure where appropriate. Before each treatment decision, patients were completely re-staged so that all re-assessed variables (i.e. liver function, tumor characteristics, therapy) were studied as TV parameters. A TV multivariable survival model was then used to evaluate the survival benefit of each treatment strategy (liver transplantation=LT, liver resection=RES, ablation=ABL, intra-arterial therapy = IAT, Sorafenib=SOR) over best supportive care (BSC). The survival benefit of HCC treatment was described as hazard ratio (HR), 95% confidence interval (CI), using BSC as reference value.

Results: The 6191 treatment strategies were: LT=155, RES = 589; ABL 1728; IAT=2184; SOR=708; BSC=827.
In the TV multivariable survival model, treatment strategy showed an independent effect on survival. The TV survival benefit of different therapies over BSC was: LT = 0.29 (0.24-0.36); RES =0.30 (0.25-0.36); ABL 0.38 (0.33-0.43); IAT = 0.51 (0.45-0.57); SOR = 0.78 (0.67-0.90).
Other significant variables in the multivariable survival model resulted: female gender, lnAFP (TV variable), and Italian Liver Cancer (ITA.LI.CA) stage (TV variable).

Conclusion: We demonstrated a decreasing TV survival benefit from surgical to systemic therapies in a large Italian cohort of HCC patients. This TV therapeutic hierarchy was independent from patient, liver function, and tumor characteristics.

Figure:
The concept of “therapeutic hierarchy” is reported in association with the ITA.LI.CA simplified staging for treatment allocation derived from the last ITA.LI.CA study.
According to this approach, treatment choice is partially independent from the disease stage. In fact, for stages 0 to B2, all therapeutic solutions are available in a hierarchical order (from LT to BSC); for stage B3 only LT is contraindicated due to an unacceptable risk of HCC recurrence; for stage C, only systemic therapy and BSC are suggested; for stage D, LT and BSC are the only possible therapies.



Abbreviations. CPS, Child Pugh score; PST, performance status; VI, vascular invasion; Intrahep, intra-hepatic; Extrahep, extra-hepatic.

P01-08YI Galactose conjugated PPI dendrimers for liver targeting

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Background and aims: Cancer therapy needs site-specific drug delivery to the affected cells and should avoid affecting the healthy cells. Liver is the prominent organ of the body and has Asialoglycoprotein receptor expression.The research aimed to develop and characterize dendrimer based drug delivery providing enhanced therapeutic potential of anti-cancer agent (doxorubicin) by effective targeting to liver cells.

Method: The 5.0 G Dendrimers were synthesized by divergent method. Ethylene diamine was the core material and Acrylonitrile the branching unit. Synthesis was performed on the basis of two stepsie Double Michael addition and Catalytic hydrogenation of nitriles to primary amines. The dendrimers were confirmed by FTIR, NMR and Mass spectroscopy and then conjugated with galactose. The shape and size were characterized by Transmission Electron Microscopy (TEM), drug loading efficiency, In-vitro drug release and stability studies. The ex-vivo studies constituted Hemolytic toxicity study. The in-vivo studies were performed on albino rats and Pharmakokinetic parameters were studied, also Biodistribution Studies were done to access doxorubicin level attained in different organs.

Results: Thus Galactosylated PPI dendrimers showed high doxorubicin loading, sustained release and excellent biocompatibility as evident by low hemolytic toxicity. Presence of ligand on dendrimer molecule, elevated receptor mediated binding thereby targeting higher concentration of doxorubicin to lung. The higher concentration of GPPI-DOX was found to be significant compared to PPI-DOX and DOX. Possibly galactose having more affinity towards asialoglycoprotein receptors of liver parenchymal cells, more amount of drug had accumulated in liver.

Conclusion: Finally, from the obtained results, it can be concluded that galactose-coated PPI dendrimers found to be most suitable for the delivery of Doxorubicin HCl. Galactose conjugation can be utilized to not only target asialoglycoprotein receptors of liver, but also to reduce hemolytic toxicity associated with the amine terminated PPI dendrimers. Furthermore, this delivery system could reduce the drug associated toxic effects by selectively targeting the hepatoma cells. Carbohydrate coated dendrimers have proved well their applicability in drug delivery to the liver, especially. The present approach can also be modified in future using different sugars other than galactose. The hurdles of dendrimer’s toxicities can be best treated with use of such molecular tailoring only in the future.

P01-09 Major impact of personalised dosimetry using 90Y loaded glass microspheres SIRT in HCC: Final overal survival analysis of a multicentre randomized phase II study

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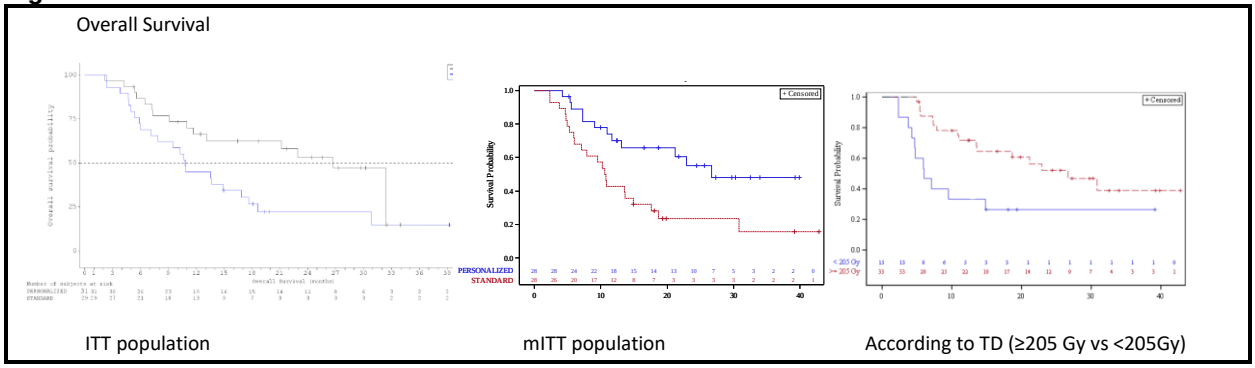
Background and aims: ^{90}Y loaded microsphere SIRT (radioembolization) is a treatment option in advanced HCC. However, no personalized dosimetric endpoints are currently used. The goal of this study was to compare the efficacy of ^{90}Y loaded glass microsphere SIRT in HCC using a standard versus a personalized dosimetric approach.

Method: DOSISPHERE-01 was a multicenter, randomized phase 2 trial in unresectable but non metastatic HCC patients with at least one tumor ≥7cm. Treatment arm was randomly assigned (1:1) to standard dosimetry arm (SDA), with a goal to deliver 120±20Gy to the treated volume or to personalized dosimetry arm (PDA) with a goal to deliver at least 205Gy to the index lesion. The primary endpoint was the response rate (RR) of the index lesion according to EASL criteria. Secondary endpoints included dose response evaluation, safety and overall survival (OS).

Results: Sixty HCC patients were randomized (PDA 31, SDA 29, intent to treat population-ITTP-), and 56 treated (28 in each arm, mITT population). Main patients and tumor characteristics were, in the PDA vs the SDA : BCLC C 87% vs 89% (ns), portal vein invasion 64.5% vs 72.4% (ns) and mean tumor size 10.4±2.6cm vs 10.9±3.1cm (ns). RR was significantly increased in the PDA versus the SDA, in the ITTP, respectively 64.5% versus 31% (p=0.0095) as in the mITTP respectively 71.4% versus 35.7% (p=0.0074). Median OS was significantly increased in the PDA versus the SDA, in the ITTP, respectively 26.7m (CI 95%:11.7-NR) versus 10.6m (CI 95%:6-16.8), p=0.0096, HR=0.421 (95%CI:0.215-0.826), p=0.0119, as in the mITT population, respectively 26.7m (CI 95%:11.6- NR) versus 10.7m (CI 95%:6.0-14.8), p=0.006. Median OS was 26.7m (CI 95%:13.5-NR) versus 6.0m (CI 95%:3.8-14.9) for the patients who received a tumor dose ≥205 Gy or <205 Gy respectively, p=0.0106, HR=0.336 (95%CI:0.154-0.735), p=0.0063**.** Treatment-related clinically relevant hepatic ≥grade 3 AEs were observed in 5.7% and 14.2% of the patients of the PDA and SDA arms, respectively, (p=ns).

Conclusion: MAA SPECT/CT based personalized dosimetry is safe and dramatically increased RR and OS of HCC patients. These results question the interpretation of all phase 3 trials of SIRT designed without personalized dosimetry in HCC.

Figure:



P01-11YI Mitochondriotropic carbon nanotubes for efficient tumor targeting

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Background and aims: Cancer is the uncontrollable growth of cells which are devoid of apoptosis. The project aimed to develop Ligand mediated tumor targeting via carrier systems. Multiwalled carbon nanotubes(MWCNTs) were selected for mitochondrial targeting as it has Central role in Apoptosis, there are Multiple activation pathways and the tumour growth depends on energy & availability of mitochondriotropics. Rhodamine-123 is shown to be rapidly taken up by mitochondria in living cells and serves a triple purpose. MWCNTs are the choice of delivery system as it directly enters into the cell without passing through endo-lysosomes, have large inner volume, have distinct inner and outer surfaces & have ability to enter cell by spontaneous mechanism. Thus, proposed work envisages Rhodamine-123 conjugated Paclitaxel loaded *functionalized*-CNTs to provide an enhanced cell permeation and mitochondrial in order to enhance mitochondrial availability of Paclitaxel.

Method: Raw MWCNT were procured and were purified, oxidized & then conjugated with rhodamine-123 by carbodimide method. MWCNT's were characterized *in-vitro* for shape & size by Scanning(SEM) & Transmission Electron Microscopy(TEM), FTIR analysis, X-ray diffraction and zeta potential determined. Stability studies were performed at exaggerated conditions along with Hemolytic Toxicity Study. Cell Cytotoxicity Study-MTT Assay was done using Hela cell lines. Mitochondrial localization was determined by CLSM study. *In-vivo* study comprised of determining distribution of drug in various organs by fluorescence microscopy.

Results: The CNTs showed high paclitaxel loading, sustained release, and excellent biocompatibility as evident by *in-vitro* drug release and low hemolytic toxicity. MTT assay against HeLa cell lines suggested the potential anticancer activity of developed system. Confocal microscopic study suggested that mitochondrial specific localization of Rhodamine-123 conjugated MWCNTs in HeLa cells.

Conclusion: Thus, we concluded that Rhodamine-123 conjugated Paclitaxel loaded *f*-CNTs system have potential to provide an enhanced cell permeation and mitochondrial localization for effective tumour chemotherapy.

P01-12YI Hepatic uptake index in the hepatobiliary phase of Gd-EOB-DTPA-enhanced magnetic resonance imaging estimates functional liver reserve and predicts post-hepatectomy liver failure

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[1]Humanitas Clinical and Research Centre - IRCCS, Department of Hepatobiliary and General Surgery, Rozzano, Italy, *[2]Humanitas Clinical and Research Center – IRCCS, Department of Radiology, Rozzano, Italy*, *[3]Humanitas University, Department of Biomedical Sciences, Pieve Emanuele, Italy*
Email: bruno.branciforte@humanitas.it

Background and aims:
Recent evidence suggests that gadolinium-ethoxybenzyl-diethylenetriamine penta-acetic acid-enhanced magnetic resonance imaging (Gd-EOB-DTPA MRI) may be used to evaluate liver function. We assessed whether the signal intensity of Gd-EOB-DTPA MRI may be used to predict functional liver reserve and post-hepatectomy liver failure (PHLF) in patients undergoing hepatectomy for liver tumors.

Method:
We retrospectively analyzed 137 preoperative Gd-EOB-DTPA MRIs of patients undergoing hepatectomy. Mean signal intensity of liver (L20) and spleen (S20) were measured on T1-weighted single-breath-hold 3D fat-saturated gradient-echo sequences acquired 20 minutes after Gd-EOB-DTPA administration.
The hepatocellular uptake index (HUI) of liver volume (VL) was calculated with the following formula VL[(L20/S20)-1], and was tested with several clinical score systems for liver diseases and to the occurrence of PHLF.

Results:
Patients with unhealthy liver had significantly lower values of HUI in comparison with those with normal function. This was found for MELD score ≤9 vs. >9 (p=0.0488), BILCHE score ≤2 vs. >2 (p=0.0208), ALBI grades (p=0.0357) and Humanitas score ≤6 vs. >6 (p=0.0311). HUI was significantly lower in those patients with PHLF (p=0.001). Receiver operating characteristics curve analysis revealed valuable HUI ability in predicting PHLF (AUC=0.84; 95%CI=0.71-0.92; p<0.001), with a cutoff value of 574.33 (98% sensitivity; 83% specificity).

Conclusion:
HUI measured on preoperative Gd-EOB-DTPA MRI identifies patients with unhealthy liver and predicts PHLF. This index could be used to discriminate those patients at higher risk of complications after hepatectomy.

P01-13YI DNA damage response protein checkpoint kinase 2 (CHK2) links chromosomal instability to cellular metabolism in hepatocellular carcinoma (HCC)

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Background and aims: Chromosome mis-segregation can cause DNA damage and induce DNA damage response (DDR). Although known as an energy-dependent process, mechanisms linking DDR to HCC cellular metabolism are unknown. Here, the involvement of CHK2, a central effector of DDR, in energy expenditure, mitochondrial functions and glycolysis was investigated.

Method: Extracellular vesicles-associated total mRNA was extracted from blood of patients with HCC (n=22), cirrhosis(n=14)and 20 healthy subjects. Steady-state metabolomic profile was performed by 1H-NMR spectroscopy in patients and cell cultures. Expression of pyruvate kinase M2 (PKM2), succinate dehydrogenase (SDH), CHK2 and γ-H2AX was evaluated by IHC in a HCC transgenic model. HuS, a human hepatocyte cell line immortalized with TERT, Huh7 and colon carcinoma cell line HCT116 were used and Illumina RNAseq was performed. Glycolysis and O2 consumption was quantified by Seahorse XF-96 analyser. Intracellular ATP was quantified by FRET-based biosensor and quantification of glycolytic/TCA cycle intermediates by trace experiments. Mitochondrial functions were assessed by TMRM.

Results: HCC patients have increased CHK2 mRNA content in extracellular vesicles which was associated with increased glycolytic metabolites. This was confirmed in a transgenic HCC model with elevated expression of PKM2 and SDH in neoplastic lesions associated with a nuclear upregulation of γ-H2AX/CHK2. During mitosis, PKM2 and phosphoglycerate kinase 1 co-localize with CHK2 and CHK2 controlled PKM2 and SDHA expression, whilst intervening with mitochondrial functions confirmed by SDH-dependent ROS production. RNAseq data provided significant changes in metabolic pathways. Cells with high levels of γ-H2AX and CHK2 exhibited increased extracellular acidification and O2 consumption rate. Unstable cells significantly rely on glycolysis for ATP production due to a defective function in mitochondrial ATP production; abolished by CHK2 knockdown. CHK2 and γ-H2AX expression promote glycolysis and succinate oxidation in unstable cells marked by increased glucose oxidation through the TCA cycle. Mitochondrial TMRM fluorescence confirmed that CHK2-controlled SDH expression is key in sustaining Δψm hyperpolarization and ROS production.

Conclusion: These data demonstrate that DNA damage and CHK2 exert an unprecedented recognized control of mitochondrial function and indicate that CHK2 is a pivotal mediator in linking DDR to cellular metabolism and mitochondrial functions.

P01-14 Integrated phenotyping of the anti-cancer immune response in HIV-associated hepatocellular carcinoma

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Background and aims: HIV-seropositivity shortens survival in patients with hepatocellular carcinoma (HCC), a leading cause of mortality in people living with HIV (PLHIV) and hepatitis. Whether HIV pre-conditions cancer immune tolerance is unknown in HCC. This is a point of greater consequence given PLHIV are excluded from trials of immune checkpoint inhibitors. We intended to verify whether HIV status influences anti-tumour immunity by evaluating the functional characteristics of the T-cell infiltrate in tumour, peri-tumoral tissue and background cirrhosis.

Method: From an international biorepository of 55 HIV-associated HCCs from 4 centres in Europe and North America, we evaluated the expression of programmed cell death ligands 1 and 2 (PD-L1/2) in tumour and immune cells at a 1% cut-off. We explored their relationship with functional characteristics of the T-cell infiltrate (cytotoxic, regulatory and helper T-cell function) in tumoral, peritumoral and cirrhotic tissue. Immuno-pathologic features were correlated with patients' characteristics including markers of HIV infection.

Results: Of the 55 patients, 41 (85%) were male and had a median age of 52 years (range 41-64). Hepatitis C virus co-infection was the leading risk factor for HCC (n=46, 90%). Most patients were of Barcelona Clinic Liver Cancer (BCLC) stage 0/A (n=40, 85%), Child-Pugh A (n=44, 86%), had median alfa-fetoprotein (AFP) values of 11 ng/ml (range 2-6536), undetectable HIV viral load (n=31, 84%), and a median blood CD4+ cell count of 428 cells/mm3. Patients were treated with liver transplantation (n=39, 71%) or resection (n=12, 22%). We observed tumoral PD-L1 expression in 24/55 (52%) and PD-L2 expression in 13/55 (28%) patients respectively. PD-L1 was frequently co-immunoexpressed in CD4+FoxP3+ (49.0 vs. 8.2 cells/mm2, p=0.002) and CD8+PD-1+ (40.8 vs. 12.3 cells/mm2, p=0.016) in tumour-infiltrating lymphocytes (TILs). This was not the case in PD-L2. PD-L1$^+$ HCCs had higher CD4$^+$FoxP3$^+$ TIL density compared to PD-L1$^-$ tumours (40.8 vs. 12.3 cells/mm2, p=0.014). PD ligands expression, CD4$^+$FoxP3$^+$ and CD8$^+$PD-1$^+$ cell density were independent of parameters reflective of HIV infection severity including peripheral blood CD4$^+$ count and HIV viral load. Peripheral CD4$^+$ counts positively correlated with CD4$^+$FoxP3$^-$ T-helper density in tumoral (r=0.38, p=0.032) and peri-tumoral cores (r=0.45, p=0.009), but not in surrounding cirrhosis. PD-L1 or PD-L2 expression was independent of patients' Child-Pugh class, AFP levels and did not predict for survival.

Conclusion: PD-L1 expression drives anti-tumour tolerogenesis in PLHIV with HCC, where prevalence of PD-L1 positivity (52%) is two-fold higher compared to historical HIV-negative controls (17%). Peripheral CD4$^+$ count is an appealing surrogate for effective T-helper infiltration in tumour and may guide the development of immunotherapy in HIV-associated HCC.

P01-15 Prognostic significance of endocan and VEGF in the prediction of hepatocellular carcinoma in patients with compensated chronic viral hepatitis C-related cirrhosis

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Background and aims: The predictive factors of the development of hepatocellular carcinoma (HCC) are not so well known in patients with hepatitis C-related compensated cirrhosis, in whom etiology-based therapy is difficult. A total of 146 patients with compensated cirrhosis, portal hypertension and no varices were included in a prospective observational study. The aim of this study was to identify predictors of the development of HCC, including endocan, vascular endothelial growth factor (VEGF), parameters indicative of liver insufficiency and portal hypertension in patients with compensated chronic viral hepatitis C-related cirrhosis with and without esophageal varices (Baveno stages 1 and 2).
Method: Baseline laboratory tests, ultrasound, endocan and VEGF measurements were performed. Patients were followed prospectively every three months until development of varices or variceal bleeding. The endpoint was HCC development according to standard diagnostic criteria. Through multivariate Cox regression, a prognostic index (PI) was developed and tested in an validation cohort (n = 40).
Results: In a median follow-up of 58 months 18/146 (12.6%) patients developed HCC. Cox regression analysis identified endocan (hazard ratio (HR) 1.11; 95% confidence interval (CI): 1.05-1.17) and VEGF (HR 0.42; 95% CI: 0.22-0.82) as independent predictors of HCC development. Using a cut-off level of 2.7, the PI [according to the following formula: 4 + (0.12 × endocan - 0.8 × VEGF)] was able to distinguish two populations of patients with very different risks of HCC development in both the exploratory and validation cohorts. A time-dependent ROC curve identified endocan as the best predictive variable.
Conclusion: Endocan and VEGF are independent predictors of HCC development in patients with hepatitis C-related compensated cirrhosis irrespective of the existence of varices.

P01-16YI Developing a bio-mimetic 3D model for hepatocellular carcinoma and the stromal micro-environment

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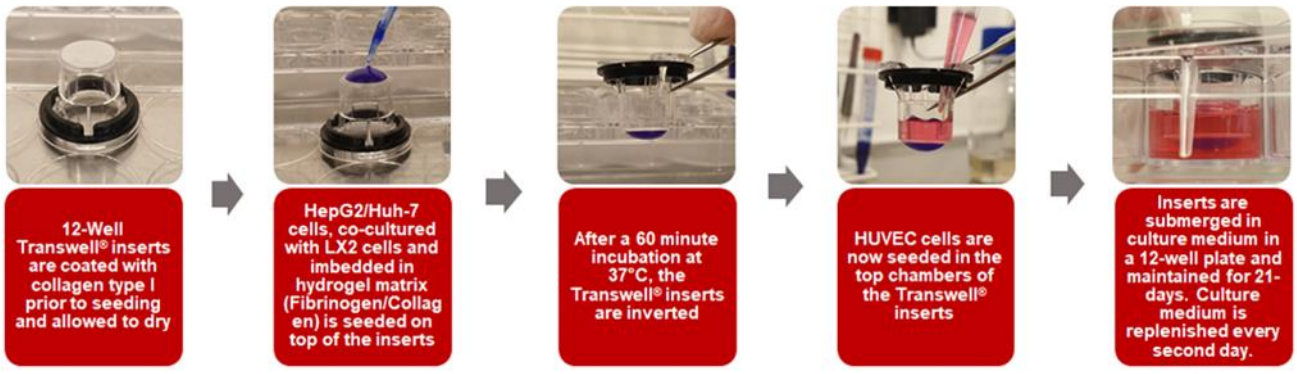
Background and aims: Hepatocellular carcinoma (HCC) is a primary liver tumour that develops in the wake of chronic liver disease. Chronic liver disease and inflammation leads to a fibrotic environment that actively supports and drives hepatocarcinogenesis. A key player in the progression of this fibrotic environment to HCC is the activated hepatic stellate cell. Insight into hepatocarcinogenesis in terms of the interplay between the tumour stroma micro-environment and tumour cells is thus of considerable importance. Three-dimensional (3D) cell culture models are proposed as the missing link between current *in vitro* two-dimensional cell culture models and *in vivo* animal models to study HCC. Our aim was to design a novel 3D model that allow us to mimic a HCC model with accompanying fibrotic stromal compartment and vasculature. Allowing us to study both endocrine and paracrine signalling within the context of a 3D environment. Physiological relevant hydrogels such as collagen and fibrinogen will be incorporated in ratios that mimic the bio-physical properties of the tumour ECM. Recapitulating not only the tumour stroma interactions, but also the influence of the ECM stiffness, which is an active mediator of cell interactions, tumour growth and metastasis.
Method: Our model attempts to recreate the sinusoidal structures during cirrhosis and HCC in a Transwell™ system, depicted in the figure below. The physical and mechanical properties of these ECM-hydrogels engrafted with cells was determined by rheology. Cell viability and drug response was evaluated in both a 2D co-culture and our 3D model by means of a known chemotherapeutic agent, Doxorubicin (0.5mM, 1mM and 1.5mM), for a period of 72h.
Results: Preliminary data indicate that this model is viable for 25-days, while giving rise to metastatic tumour nodules after 17 days in culture. Rheology results show that we can successfully mimic the bio-physical properties of a cirrhotic liver. Furthermore, our model showed reduced sensitivity to a known chemotherapeutic compared to traditional 2D cultures.
Conclusion: We have successfully developed a 3D model for studying tumour-stroma interactions in hepatocellular carcinoma. Overall, our results indicate that this 3D model is more representative of the *in vivo* situation compared to traditional 2D cultures. Specifically, the 3D tumour model showed a decreased response to chemotherapeutics, therefore mimicking drug resistance typically seen in HCC patients. In addition, our model gave rise to metastatic tumour nodules. This could provide an interesting new platform to study multifocal HCC or to identify mechanisms that contribute to early stages of metastasis. We have also identified the optimal hydrogel composition that mimics the bio-physical properties of a cirrhotic liver.
Figure:
Figure 1: *Graphical depictions of the creation of our 3D HCC Transwell™ model.*



P01-17 Identification of a proteomic signature in advanced hepatocellular carcinoma predicting response to sorafenib

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Background and aims: Hepatocellular carcinoma (HCC) is the most common liver cancer usually developed on cirrhosis. Because only 50 to 70% of cancers are diagnosed late, its prognosis is extremely poor; that's why advanced HCC can only be treated with regional or systemic palliative therapies. In most countries, sorafenib is approved as the standard first-line therapy for advanced HCC. Recently, the significant increase in therapeutic possibilities raises a new important problem to know which treatment to give to which patient. Biomarkers predicting sorafenib efficacy could assist in identifying the minority of patients who are likely to benefit from the treatment and would allow patients to be redirected to other treatment options.
Method: We used a technological process combining laser capture and mass spectrometry analysis that we previously developed in the laboratory to thoroughly analyze tumor proteomic profiles (Henriet *et al*, Hepatology 2017). This method is compatible with the analysis of formalin-fixed and paraffin-embedded tissues (FFPET) and with very small quantities of material such as diagnostic biopsies (1mm² on a 5µm thick cut). We analyzed protein expression deregulations between tumor and non-tumor tissue from diagnostic biopsies before treatment. We thus compared the tumor proteome of 6 good responder patients (radiological tumor regression >30% and/or decrease in AFP > 50%) to 7 bad responder patients (radiological tumor progression >20%). We then performed functional validation in HCC cell lines monitoring cell viability with the Incucyte Zoom System (Essen Bioscience).

Results: We have identified a proteomic signature to distinguish good responders from bad patients from their diagnostic biopsies. Among the proteins of this signature we revealed that chaperone and oxidative stress pathways could be theranostics indicators. Indeed, the upregulation of these proteins could reduce sensitivity to sorafenib treatment in patients with poor response. To validate this hypothesis, we selected the transketolase (TKT) protein with the most recurrent overexpression in patients with poor response. TKT depletion significantly increases the effect of Sorafenib in HUH7 cells, while its overexpression reduces its effect which confirms the relevance of our theranostic signature.

Conclusion: Through this study we demonstrate the tumor proteomic profile analysis relevance in precision medicine for advanced HCC patient's management. This result highlights biopsies potential and the added value to include mass spectrometry based proteomic analysis into the HCC diagnostic process for theranostic.

P01-18YI The volume of enhancement of disease (VED) predicts the early response to treatment and overall survival in patients with advanced hepatocellular carcinoma treated with sorafenib

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Background and aims: The response of patients with advanced HCC treated with sorafenib is still unpredictable. We analysed the predictive value of the Volume of Enhancement of Disease (VED), a new radiologic parameter based on the arterial enhancement coefficient (ΔArt%) in computed tomography, in the early evaluation of the response to sorafenib in patients with advanced HCC.
Method: We included patients with advanced hepatocellular carcinoma (HCC) who underwent a multiphase enhanced CT (multidetector Somatom Sensation 64 CT scan) before (T0) and after 60-70 days of therapy with sorafenib (T1), enrolled between 2012–2016,. The same target lesions utilised for the assessment of response were used for the calculation of size and for the calculation of VED (volume lesion x ∆art% / volume lesion). We compared these values at T0 and T1 in patients with a clinical benefit (CB, the composite of complete response, partial response and stable disease) from therapy or with progressive disease (PD). Survival probability was evaluated in the study population and in the different subgroups of patients, based on tumor size and VED, but also on ancillary imaging findings and blood chemistries.
Results: Thirty-two patients with advanced HCC treated with sorafenib (25 men, 7 women, mean age 65.8 years) were selected. At T1 8 patients had CB (1 partial response, 7 stable disease) and 24 had PD. VEDT0 was >70% in 8/8 CB patients compared to only 12/24 patients in the PD group (P=0.011). In CB patients, but not in PD, VEDT1 values were significantly lower than those at T0 (p=0.018). No significant differences in the ancillary imaging findings were found between the two time points. Patients with VEDT0 >70% showed a significantly higher median survival than those with lower VEDT0 (506 vs. 266 days, p=0.032). Patients with VEDT0 > 70% and alpha-fetoproteinT0 ≤ 400 ng/ml had a significantly longer survival than all other combinations of the two biomarkers (median survival: 582 days vs. 208-213 days for the other combinations of the two biomarkers).
Conclusion: In patients with advanced HCC treated with sorafenib, VED is a novel and simple radiologic parameter obtained by contrast-enhanced CT, which could be helpful in selecting patients who are more likely to respond to sorafenib therapy, and with a longer survival. Patients with baseline VED value > 70% and alpha-fetoprotein ≤ 400 ng/ml showed longer survival.

P01-19YI Development of diet-induced NASH/HCC model in Sprague Dawley rats

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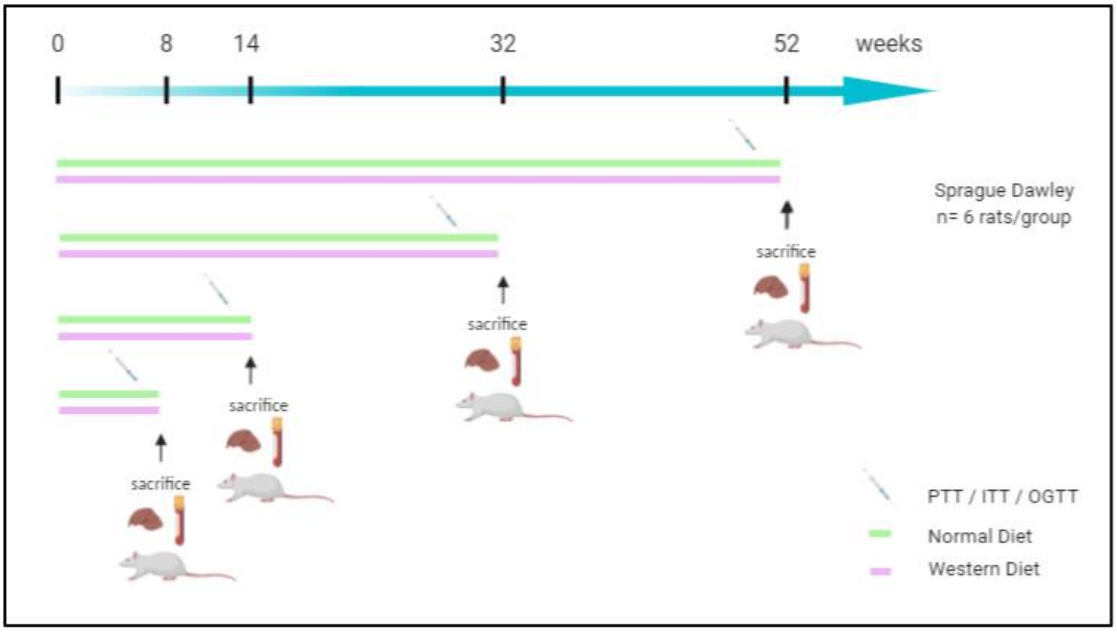
The incidence of nonalcoholic steatohepatitis (NASH) is increasing with the simultaneous prevalence of obesity, particularly in western countries. However, carcinogenesis mechanisms induced by NASH are poorly understood due to the lack of adequate animal models. Nowadays, animal models, mainly mice, are genetically modified or exposed to carcinogen-inducing chemicals and don't replicate the full range of liver disorders associated to NASH, especially HCC, probably due to short period of treatment and young age of animals.
In this project, we want to establish a new diet-induced NASH/HCC animal model, which mimics as close as possible the pathophysiology of NASH in human patients and reproduce liver microenvironment that lead to HCC development in an immunocompetent model.
Sixty 7-weeks-old male Sprague Dawley rats were fed by a normal diet (ND) or a western diet with ad libitum consumption of glucose and fructose (WD) in physiological relevant concentration. Three different time points were analyzed (8, 14, and 32 weeks). One week before sacrifice, metabolic disorders were assessed by pyruvate tolerance test (PTT) at 8 and 14 weeks, oral glucose tolerance test (GTT) and insulin tolerance test (ITT) at 32 weeks. At each time point, blood was taken, and liver, subcutaneous and visceral adipose tissues were harvested for histological and gene expression studies.
At 8 weeks, WD fed rats developed diabetes, associated with hepatic neoglucogenesis deregulation. They displayed obesity, associated with an increase of visceral adipose tissue, hypertriglyceridemia and abnormal triglyceride accumulation in the liver, and a tendency to hepatomegaly. Hepatic lipogenic genes, such as Pparγ and Scd1, were upregulated in WD group. At 8 weeks, no differences were observed in cell proliferation and inflammatory related genes. At 14 and 32 weeks, WD rats continued to gain excessive weight compared to ND rats. However, no change between WD and ND groups were observed regarding metabolic disorders and liver or adipose tissue pathologies, except for 32 weeks WD fed rats which showed significant increase in the visceral adipose tissue weight.
Our WD rat model developed metabolic syndrome at 8 weeks of diet (obesity, diabetes, hyperlipidemia). Unexpectedly, even though 14 and 32 weeks WD rats continued to display significant obesity compared to ND rats, glucose and lipid homeostasis was restored through compensatory mechanisms and behavior, including calorie intake decrease. Interestingly, visceral adipose tissue from 32 weeks WD rats was heavier than control rats. Thus, we extended our experiment to 52 weeks of WD feeding animal, expecting that compensatory mechanisms will be overwhelmed, leading to a tissue environment favorable to HCC development.
Moreover, further analysis to understand the compensatory mechanism needs to be done to shed the light on a new line of therapy in NASH.

Figure:


0
8
14
32
52
weeks
Sprague Dawley
n= 6 rats/group
sacrifice
sacrifice
sacrifice
sacrifice
PTT / ITT / OGTT
Normal Diet
Western Diet

P01-20YI Portal hypertension and sequential bilobar treatment increase risk of hepatic decompensation in patients with hepatocellular carcinoma treated with 90Yttrium radioembolization

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Background and aims: Transarterial Radioembolization (TARE) is widely used in patients with hepatocellular carcinoma (HCC). It has been suggested that TARE could increase the risk of hepatic decompensation and development of portal hypertension (PH). Liver function has been demonstrated to be associated with overall survival in HCC. Our objective was to identify the incidence of hepatic decompensation and associated factors.

Method: 63 consecutive patients treated with TARE between February 2012 and December 2018 were included. Clinical, analytical and tumour characteristics before the treatment and after 3 months were analysed. Hepatic decompensation was considered as the development of 1 or more of the following: clinical ascites, hepatic encephalopathy, portal hypertension bleeding, MELD increase >3 with a punctuation >9 or Child-Pugh's increase. Clinical significant PH (CSPH) was assumed when a HVPG>10mmHg, there were esophageal varices or platelets <85.000.

Results: Mean age was 67,7 years (range 42-90) with 88.9% men, mainly with non-early HCC: 10 patients (15.9%) were in BCLC stage A, 32 (50.8%) were in B stage and 21 (33.3%) in C stage. 58.7% of the patients had received previous treatments. The majority was cirrhotic (76.2%) with compensated liver function (93.6%). The most frequent etiology was hepatitis C (33.3%), and hepatitis C and alcohol (23.8%). The treatment was unilobar or segmentary in 68.2% and bilobar in the rest. Mean administered dose was 2.12 GBq (0.47-11). Median progression-free survival was 5 months. Median overall survival was 16 months. Almost a third of the patients (28.6%) presented hepatic decompensation in the following 3 months without correlation with tumour progression (18 patients, 32.6%). In the univariate analysis the related variables associated with hepatic decompensation were: presence of cirrhosis (OR 8.85 (1.06-73.25, p=0.014)], bilobar treatment [OR 3.1 (0.96-9.96, p=0.056)], previous clinical decompensation [OR 4.87 (1.00-23.27, p=0.043)] and presence of CSPH [OR 12.50 (2.53-61.80, p=0.000)]. In the multivariate analysis only bilobar treatment [(OR 3.86, 0.96-15.41 p=0.057] and CSPH [(OR 14.18, 2.66-75.49 p=0.000)] were associated with hepatic decompensation.

Conclusion: TARE in HCC patients could affect liver function and risk of decompensation especially in patients with CSPH or with bilobar sequential treatment. These findings could be useful in the selection of TARE candidates.

P01-21YI Targeting tumor-initiating cells as an effective approach to overcome sorafenib resistance in hepatocellular carcinoma

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Background and aims: Development of chemoresistance is frequently observed in the majority of HCC patients. Evidence suggests that cancer stem cells (CSCs) may contribute to the acquisition of resistance in many solid tumors, but their exact role in this process for HCC remains to be defined. Here, we evaluate the importance of CSCs in the development of resistance and relapse formation after exposure to sorafenib in HCC and define concomitant adaptive molecular targets.
Method: Four HCC cell lines and two primary HCC isolates were exposed to sorafenib for a total of 14 days. The treatment effects on CSCs were estimated by sphere forming capacity *in vitro* and tumor-initiating potential *in vivo*, as well as the side-population (SP) approach. Expression of key oncogenic and CSC markers, such as EpCAM, CD133 and ABCG2 transporter, were assessed by qRT-PCR and flow cytometry. Furthermore, whole transcriptome analyses were performed across the cell lines and identified potential targets which were further validated by western blot and administration of specific inhibitors.
Results: Treatment effectively reduced oncogenic properties in all investigated HCC cells. However, sustained anti-proliferative effect after treatment was observed in three cell lines, while initial treatment effect in other lines was subsequently followed by rapid re-growth thereby mimicking the responses observed in patients. While anti-oncogenic effects in sensitive cell lines were associated with significant reduction in sphere forming and tumor-initiating capacity, CSC marker EpCAM as well as SP cells, resistant cell line showed transient increased in CSC properties. Acquired resistance to the drug uniformly developed in cell lines suggesting that common molecular mechanisms might be operative. These adaptive molecular changes involved signaling pathways known to be associated to cell survival, proliferation and cell cycle regulation (RAS, AKT, MYC, P53), as well as angiogenesis (VEGFR, PDGFR). Furthermore, the resistant cell lines showed compensatory upregulation of key oncogenic molecules such as EGFR, multidrug resistance ABC transporters as well as YAP. Conclusively, combined treatment including sorafenib and specific YAP inhibitor showed beneficial effects in resistant cell lines which resulted in complete response to the therapy.
Conclusion: Our model recapitulates features of drug resistance observed in human HCC patients. Resistance to sorafenib therapy might be fueled by transient expansion of CSCs. Therefore, specific targeting of CSCs as well as pro-oncogenic compensatory signaling pathways might be an effective therapeutic strategy to overcome resistance in HCC.

P01-22Y1 Characterisation of a plasmid-based mouse model of liver cancer with concomitant liver injury

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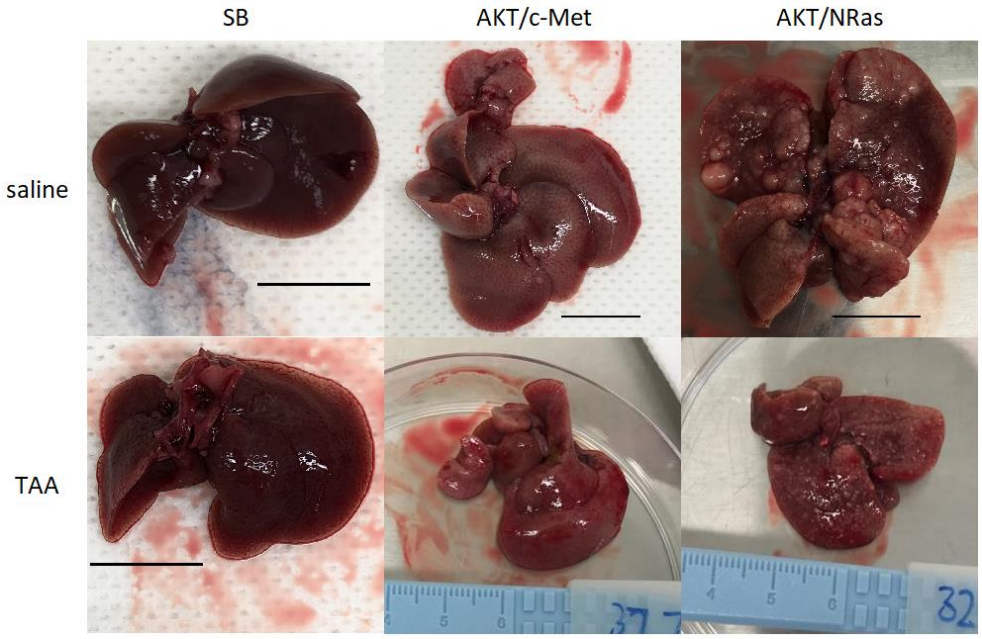
Background and aims: Liver cancer is characterised by a poor prognosis and growing disease burden worldwide. A novel type of liver cancer model utilises *in vivo* transfection using plasmids encoding oncogenes and Sleeping Beauty transposase (SB). Hydrodynamic tail vein injection (HTVI) is an effective means of expressing plasmids in the liver. However, such models typically do not recapitulate the chronic injury and fibrosis commonly seen in human liver cancer. This study aims to examine a plasmid-HTVI liver cancer model combined with concomitant liver injury.

Method: Plasmids for myr-AKT (AKT), NRasV12 (NRas), c-Met and SB were previously described by Ho et al. (2012) and Hu et al. (2016). 14-15 week-old male C57BL/6J mice were given an HTVI of SB plasmid alone ('negative control'), or with one of AKT, NRas or c-Met ('single plasmid') or with AKT and NRas, or AKT and c-Met ('double plasmid'). 8-10 days post-HTVI, mice received intraperitoneal injections of thioacetamide (TAA) or saline twice per week. Mice were harvested after 10-11 intraperitoneal injections.

Results: Gross examination of livers on excision found that single plasmid groups had little or no change from respective saline or TAA negative control (mild hepatomegaly in SB/AKT groups). Double plasmid plus transposon groups showed gross hepatomegaly and nodules (SB + AKT/NRas or + AKT/c-Met). SB/AKT/NRas showed greater tumour burden than SB/AKT/c-Met. Addition of TAA altered the gross appearance of SB/AKT/NRas mice; nodules were smaller and more numerous. Histopathology of single plasmid groups found minimal change compared to negative control, except SB/AKT with diffuse steatosis. In contrast, double plasmid groups with saline displayed widespread steatosis with lesions including dysplastic nodules, regenerative nodules and bile duct adenomas. Following TAA and double plasmids, hepatocyte ballooning was widespread and the HCC that developed were significantly smaller.

Conclusion: This study found that TAA-induced liver injury significantly altered the phenotype of the double plasmid groups. Single plasmids with TAA were insufficient to cause cancer within the study timeframe (SB + AKT, + NRas or + c-Met). Chronic liver injury is an important modulator of hepatocarcinogenesis but our results are consistent with synergistic oncogene pathway activation being required for liver cancer development.

Figure:



P01-23 The role of endosonography and endosonography guided fine needle aspiration in detection of small sized liver metastasis in patients with pancreatic and gastro-intestinal malignancy

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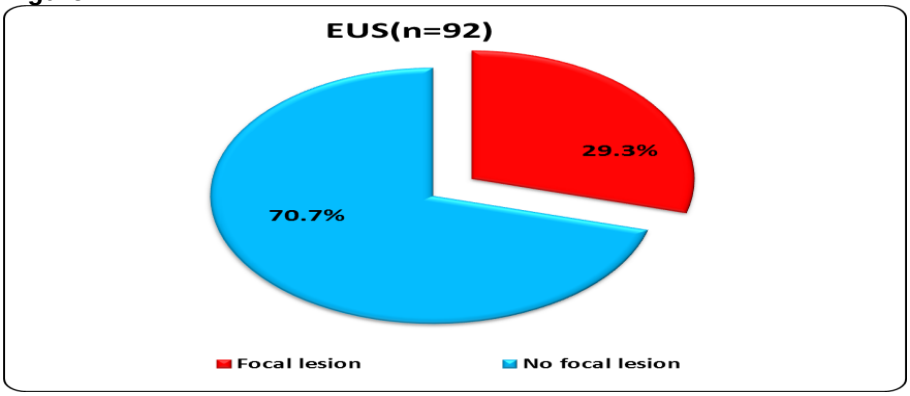
Background and aims: Liver is common site of metastasis for both pancreatic adenocarcinoma and gastrointestinal (GI) tumors. Small liver masses (< 2 cm) are not easily accessible by ultrasound or computed tomography (CT) guided percutaneous biopsy. Endosonography (EUS) allows clear visualization of liver anatomy and its vasculature. Aims: To assess usefulness of EUS and EUS-FNA in detecting small sized liver metastasis; not diagnosed by ultrasound and CT scan during TMN staging of GI and pancreatic malignancy, which can affect the management plan.
Method: This prospective comparative study included 92 cases with confirmed pancreatic, peri-ampullary and GI malignancies, who presented to faculty of medicine, Cairo university. The following labs were done: CBC, serum bilirubin, AST, ALT, ALP, GGT, serum albumin, prothrombin concentration. ALL patients were assessed by abdominal ultrasound and CT scan. Oesophago-gastro-duodenoscopy was performed in cases of GI malignancy. EUS was done for all patients, and EUS guided fine needle aspiration (EUS-FNA) was performed in 23 patients with liver focal lesions detected by EUS, followed by cytological examination.
Results: Among the 92 patients, CT scan detected 27 cases with liver focal lesions prior to performing EUS/EUS-FNA, where one of these patients was missed by EUS (25 mm in diameter focal lesion, hyperdense, single, in right lobe). While EUS detected 27 cases with liver focal lesions, and one of these cases was missed by CT (4 mm in diameter focal lesion, hypoechoic and located at segment IV). Among these 27 cases diagnosed by EUS, 4 cases were cholangitic abcesses, EUS-FNA was performed in 23 cases, revealing metastatic lesions in 21 patients (91.3 %), benign lesions in 2 patients (8.7 %) (focal fat depletion). None of the patients suffered from major or minor complications post EUS-FNA. Our study showed that EUS had 95.45 % sensitivity, 97.14 % specificity, 91.3 % positive predictive value (PPV), 98.55 % negative predictive value (NPV) and 96.74 % accuracy. CT had 95.45 % sensitivity, 91.43 % specificity, 77.78 % PPV, 98.46 % NPV and 92.39 % accuracy. And EUS–FNA, had 95.45 % sensitivity, 100 % specificity, 100 % PPV, 98.5 % NPV and 98.91 % accuracy.
Conclusion: EUS and EUS-FNA play a significant role in detecting small sized liver metastasis, and differentiating it from benign lesions, during TMN staging of GI and pancreatic malignancy, however EUS results were comparable to that of CT scan.

Figure:



P02-01 Treatment-related toxicity predicts for improved outcome in patients with hepatocellular carcinoma (HCC) treated with immune checkpoint inhibitor therapy

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Background and aims: Reversal of cancer immune-tolerogenesis with immune checkpoint inhibitors (ICI) is an emerging therapeutic modality in HCC, although the precise molecular mechanisms underscoring ICI efficacy are poorly understood. The development of treatment-related adverse events (trAE) favourably influences outcome in ICI recipients. In this multi-center study we aimed to verify its prognostic role in HCC.

Method: We established an international consortium of 9 tertiary referral centres located in Europe (n=68), United States (n=226) and Asia (n=47) to derive a prospectively maintained cohort of 341 patients who underwent ICI treatment. We tested whether the development of clinically significant trAE (ie. graded >2) predicted for improved overall response rates (ORR) and overall (OS), using time-dependent survival analyses.

Results: Of 331 eligible patients, 254 (76%) had Barcelona Clinic Liver Cancer stage C HCC, 233 were cirrhotic (70%) mostly due to Hepatitis C infection (n=129, 39%). Patients had received at least 1 line of prior systemic therapy (n=297, 90%) before anti-PD-1/PD-L1 ICI monotherapy (n=280, 85%) or combinations (n=51, 15%). Median OS was 12.1 months (95%CI 9.2-15 months) and ORR was 19% with 23 complete (6%) and 42 partial responses (13%). Overall, 133 patients (40%) experienced at least 1 trAE. Seventy were of grade >2 (21%), most frequently affecting the liver (n=24, 7%). Permanent ICI discontinuations were secondary to disease progression (n=160, 48%) or unacceptable toxicity (n=12, 4%).

Emergence of trAEs graded >2 whilst on ICI predicted for improved OS (median 19.7 versus 11.0 months, Hazard Ratio [HR] 0.32, 95%CI 0.16-0.65, p=0.001) and increased ORR (30% vs. 16%, Chi-square 5.9, p=0.01). Other univariable prognostic factors included AFP>400 ng/mL (HR 1.49 95%CI 1.1-2.0, p=0.01), Child Pugh Class (B vs. A, HR 2.74, 95%CI 1.8-4.0, p<0.0001) and geographical origin (Europe vs. Asia HR 0.50 95%CI 0.27-.092, p=0.02). Following time-dependent Cox regression analyses, the occurrence of trAEs remained a predictor of improved OS (HR 0.39, 95%CI 0.16-0.92, p=0.03) independent of Child Pugh Class (HR 1.99, 95%CI 1.18-3.35, p=0.009), BCLC stage (p=0.43), AFP levels (p=0.11), geographical origin (p=0.67) corticosteroid therapy (p=0.54) and type of immunotherapy regimen received (monotherapy vs. combination, p=0.58).

Conclusion: This is the first study to demonstrate that trAEs may influence response and survival in patients with HCC receiving ICI. Mechanistic studies highlighting the immune-biologic foundations of such relationship are warranted.

P02-02YI Response rates to direct antiviral agents (DAA) among hepatitis C virus infected patients who develop hepatocellular carcinoma following DAA treatment

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Background and aims: One of the possible explanations for the disturbingly common occurrence of *de novo* hepatocellular carcinoma (HCC) soon after treatment of hepatitis C virus (HCV) infection with direct antiviral agents (DAA) is that these patients may have been harboring hitherto hidden tumors. A clue that this might be the case would be a lower sustained viral response (SVR) rate, since patients with active HCC respond sub-optimally to DAA. We aimed to substantiate this hypothesis.
Method: We retrieved electronic medical records of all patients with benign chronic liver disease who attended the liver clinic of an Academic hospital in northern Italy to receive DAAs for hepatitis C and a) had no history of HCC; b) had received at least one DAA dose; c) had been followed-up clinically and ultrasonographically for at least six months after concluding DAA.
Results: The final study population included N.=789 patients (55% males, median age 62 years), who received either DAA regimens based on sofosbuvir (N.=370, 47%) or based on a protease inhibitor (N.=419 cases, 53%). The intention-to-treat SVR was 770/789 (97.6%); the virologic failure rate was 14/789 (1.8%). A median of 9.3 months (interquartile range 8.8–11.9) after the conclusion of antiviral therapy, N.=19/789 (2.4%) patients were discovered to have HCC. Among them, 15/19 (79%) at diagnosis had either one or two nodules while 7/19 (37%) had portal vein thrombosis. At univariate analysis, in comparison to the remaining N.=770 HCC-free patients, patients with HCC were more commonly males (84 vs. 54%, p = 0.009), obese (47 vs. 17%, p =0.002), and cirrhotics (95 vs. 35%, p <0.001) and had less commonly achieved an SVR (68 vs. 98%, p <0.001); moreover, they had a trend for being less commonly treatment naïve (58 vs 67%, p =0.051). At multivariate analysis, the independent predictors of HCC development were male sex (O.R.= 4.59, 95% C.I. 1.18-17.9, p = 0.028), cirrhosis (O.R=20.3, 95%C.I. 2.60-159.3, p = 0.004), obesity (O.R.=4.73, 95%C.I. 1.57-14.3, p = 0.006) and SVR (O.R.= 0.04, 95%C.I. 0.01-0.18, p<0.001).
Conclusion: SVR is the strongest independent predictor of development of HCC early after treatment of hepatitis C with DAA. Lack of achieving SVR should raise the suspicion of this complication, especially among HCV carriers who are male, obese, and cirrhotic.

P02-03YI Hepatotoxicity during treatment with immune checkpoint inhibitors: what happens to the liver with tumor infiltration?

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Background and aims: Treatment with immune checkpoint inhibitors (ICI) is often complicated by the development of hepatic immune-related adverse events (HIRAEs). Little is known about the risk factors for HIRAEs. We tried to assess if hepatic infiltration by hepatocellular carcinoma (HCC) and by non-HCC cancers with liver metastases could be linked to HIRAEs. Furthermore, we investigated a possible relation between HIRAEs and hepatic tumor burden.

Method: Our analysis included 76 patients treated with ICI in our center between August 2015 and May 2019. 36 patients had unresectable/advanced HCC, 40 patients had non-HCC cancers metastatic to the liver (4 renal cancer patients, 25 non-small-cell lung cancer patients, 11 melanoma patients). 56 patients received a single agent treatment with an ICI targeting the programmed cell death receptor-1 or its ligand (PD-1/PD-L1) or the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). 20 patients received a combination therapy. HIRAEs were categorized according to the Common Terminology Criteria for Adverse Events (v. 5.0).

Results: The frequency of any grade HIRAEs was significantly higher in the HCC group compared to the non-HCC group (50% vs 27%, p = 0.04), while the frequency of G3-G4 HIRAEs was similar (14% vs 5%, p = 0.41). We observed comparable rates of temporary treatment suspensions (11% vs 7.5%, p = 0.70) and permanent discontinuations (0% vs 7.5%, p = 0.24). Any grade HIRAEs were significantly more frequent in patients with three or more HCC liver nodules (p = 0.035), while this association was marginally significant in the non-HCC group (p = 0.055).

Conclusion: In our retrospective analysis, HCC patients suffered from more frequent HIRAEs compared to patients with other malignancies metastatic to the liver, while G3-G4 HIRAEs and the rates of treatment discontinuations did not differ significantly between the two groups.

Furthermore, patients with three or more hepatic nodules were more prone to develop HIRAEs, suggesting a possible role of hepatic tumor burden as risk factor for HIRAEs.

P02-04 Epidemiological trends of hepatocellular carcinoma in patients with nonalcoholic fatty liver disease in Italy. On behalf of the ITA.LI.CA. study group

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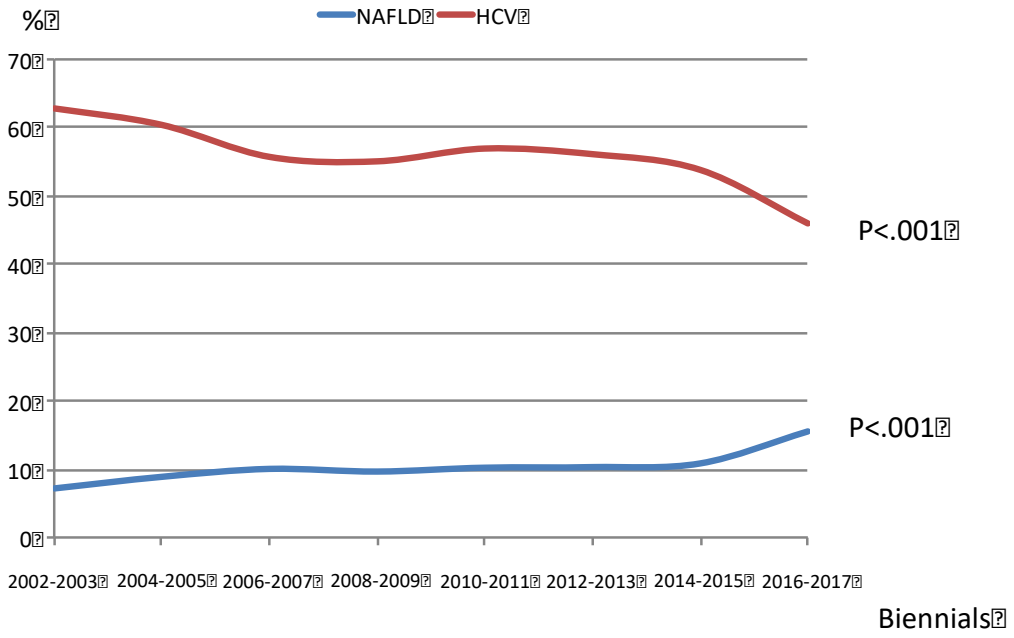
Background and aims: Non-alcoholic fatty liver disease (NAFLD) is projected to become the leading cause of hepatocellular carcinoma (HCC) in Western countries within 2025.The Italian Cancer Liver (ITA.LI.CA) database, that has collected over the last 20 years data of a huge western population of HCC patients, offers the ideal possibility to depict the epidemiological trends of NAFLD-associated HCC in the last years.

Method: We analysed 6,485 consecutive HCC patients diagnosed and enrolled from 2002 to 2017 in this multicenter Italian database. To describe epidemiological trends, the study period was divided in eight consecutive biennials (2002-2003 to 2016-2017). We analysed trends in liver disease severity, HCC stage and treatment strategies according to the HCC aetiologies.

Results: The proportion of NAFLD-HCC patients significantly increased in the study period from 7.19% in the first biennium, to 15.53% in the last ($p<0.001$). Conversely, the proportion of hepatitis C related-HCC patients significantly decreased from 62.83% to 45.96% ($p<0.001$). The proportion of hepatitis B related–HCC similarly decreased from 18.94% to 12.20% ($p<0.001$), with a sharp decrease the overall of viral aetiology ($p<0.001$). The proportion of HCC related to alcohol abuse remained stable around 16% ($p=0.183$). Cirrhosis was present in about 75% of NAFLD-HCC patients, in contrast to the near totality of other HCC patients ($p<0.001$). In addition, the proportion of cirrhosis in NAFLD-HCC patients slightly decreased from 86.67% in the first biennium to 74.7% in the last ($p=0.03$). NAFLD-HCC patients showed less often a clinically significant portal hypertension and an early tumor stage, but these differences did not impact in their potential for radical therapies and overall survival (median survival 42 vs. 44 months in NAFLD vs other HCC patients, $p=0.77$). Moreover, considering the different treatments and the ITA.LI.CA staging subgroups, no differences in overall survival were observed between NAFLD and other HCC patients.

Conclusion: The proportion of NAFLD-HCC in Italy has significantly increased from 2002 to 2017, with a progressive reduction of the association with cirrhosis (absent in about 1 out of 4 NAFLD-HCC patients). The outcome of NAFLD-HCC patients does not differ from that of the other HCC cases.

Figure:
Epidemiological trends of NAFLD and HCV aetiologies in ITA.LI.CA patients with HCC, period 2002-2017



P02-05 Immunological approach to identify predictive factors for development of hepatocellular carcinoma in patients treated with direct-acting antivirals

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Background and aims: Chronic hepatitis C virus (HCV) infection is one of the major risk factors for the development of hepatocellular carcinoma (HCC). New direct-acting antivirals (DAA) substantially improved the cure rate of HCV to above 95% but the incidence of HCV-related HCC remains high. To identify patients at risk for HCC, we investigated a cohort of patients who developed de novo HCC following DAA treatment in comparison to controls who did not develop HCC.

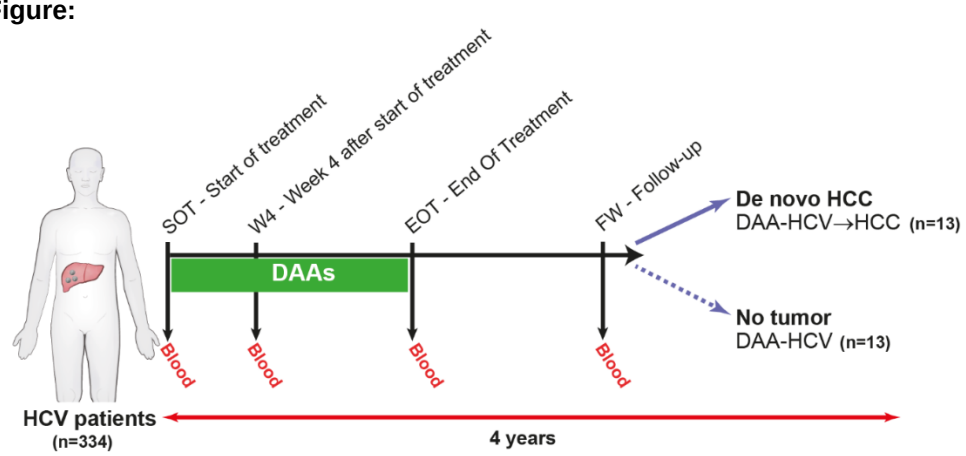
Method: Patients with chronic HCV, enrolled at department of Gastroenterology and Hepatology, CHU Grenoble-Alpes between 2014 and 2015, were treated by DAA and followed over the 4-year period (n=334). Patients with history of HCC and with the lack of SVR after DAA-treatment were excluded from future analyses. 13 patients developed de-novo HCC after DAA treatment. Matched controls were selected based on gender, age, fibrosis status and platelet counts. We evaluated serum levels of 30 immune mediators before, during, at the end and three months after DAA treatment by Luminex technology.

Results: We selected a set of immune mediators (cytokines and soluble immune checkpoints) whose levels were significantly different in DAA treated patients who developed HCC compared with controls. We observed that IL4 and IL13 levels were significantly higher in serum before DAA treatment of patients who later developed HCC compared with controls and stayed higher for each subsequent time point. Analyses of changes in levels of inflammatory cytokines during DAA treatment provided important information about HCV-induced carcinogenesis and the effects of DAAs. To verify the prognostic value of selected markers, an independent cohort of HCV-infected patients treated by DAA is currently prepared in collaboration with Irkutsk State Medical University.

Conclusion: We identified a set of possible predictive factors for risk of HCC occurrence following DAA treatment. The differences in cytokine levels were observed already at baseline of DAA treatment which confirms the existence of pro-oncogenic immune profile in these patients who later develop HCC. Such results can improve the clinical management of HCV chronically infected patients treated by DAA prior to the development of HCC. However, larger independent cohort should be used to verify the prognostic value of selected markers.

Project is supported by i) Fond de Dotation AGIR, France ii) Ligue National contre le Cancer CD38, France and iii) PHC Kolmogorov 2018-2020 France (41155RB) and Russia (RFMEFI61618X0098).

Figure:



Design of study. Patients with chronic HCV were treated by DAA and followed for HCC development over the 4-year period (n=334). Serum levels of patients who developed de novo HCC following DAA treatment (DAA-HCV→HCC, n=13) and patients who did not develop HCC (DAA-HCV, n=13) were compared.

P02-06YI Interplay of PNPLA3 and HSD17B13 variants in modulating the risk of hepatocellular carcinoma among hepatitis C virus infected patients

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Background and aims: A single nucleotide polymorphism causing a C to G change in the PNPLA3 gene (rs738409) is associated with disease severity and development of hepatocellular carcinoma (HCC) in non-alcoholic fatty liver disease; the insertion variant rs72613567:TA of the 17β-Hydroxysteroid-dehydrogenase type 13 (HSD17B13) mitigates this detrimental effect. Our aim was to evaluate if the same holds true in chronic hepatitis C virus infection (HCV).

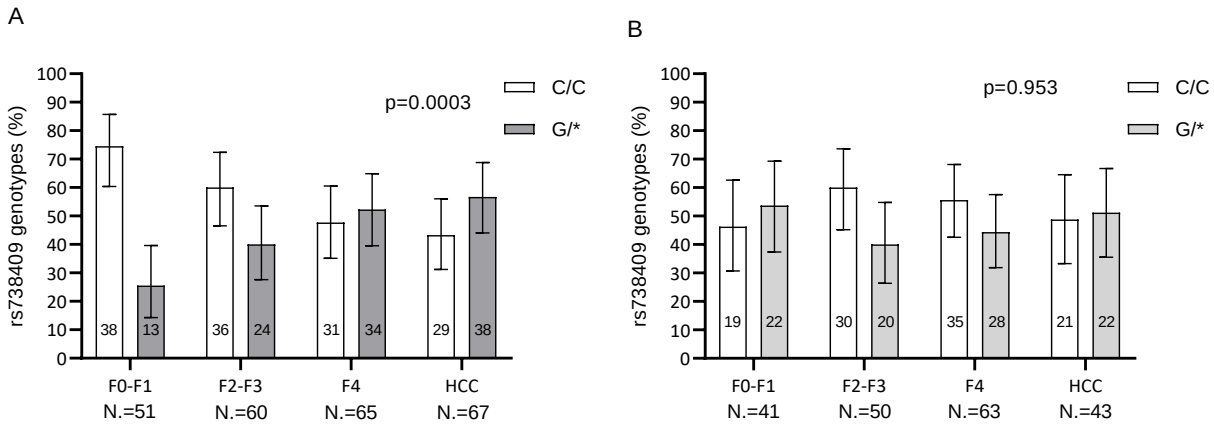
Method: With a case control retrospective study design, we selected 110 patients who developed HCC on a background of HCV infection, matching each HCC patient for sex and age (± 30 months) to three HCV infected, non-HCC patients. All participants underwent genotyping for *PNPLA3* and *HSD17B13*) gene variants. Both univariate and multivariate (conditional logistic regression) analysis of risk factors for advanced disease and HCC were performed.

Results: Carriage of *PNPLA3* G* allele was associated with a trend of progressively more severe liver disease, from mild fibrosis to significant fibrosis, cirrhosis and HCC (p=0.007). When the *HSD17B13:TA* status of these patients was taken into account, the abovementioned trend was strengthened among *HSD17B13* wild-type homozygotes, and completely blunted among carriers of the variant allele (p= 0.0003 and 0.953, respectively) (Figure, panels A and B).

In a conditional logistic regression model including diabetes and AST-to-platelets ratio index in the set of predictor variables, the unfavourable genetic profile characterized by the co-existence of mutated *PNPLA3* and *HSD17B13* wildtype was an independent risk factor for HCC (OR=2.00, CI95%: 1.23-3.26) together with history of alcohol abuse.

Conclusion: The combination of being *PNPLA3* mutated and *HSD17B13* wild-type significantly increases the risk of developing HCC among HCV-infected patients. The interplay between the two genes may explain some of the controversy on this topic, and may be exploited to stratify HCC risk in hepatitis C.

Figure:



P02-07 The NADPH oxidase NOX4 regulates lipid metabolism, which contributes to its tumor suppressor actions 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 (ROS) in signal transduction. The isoform NOX4 has been implicated in a variety of physiological and pathological processes. In recent works, we found that stable knockdown of NOX4 expression in liver tumor cells increases their proliferative capacity in vitro and enhances their tumorigenic potential in xenografts in mice, resulting in earlier onset of tumor formation and increase in tumor size (Crosas-Molist et al., Free Radic Biol Med 2014). NOX4 could also regulate other cellular processes that occur later in progression and that favor tumor metastasis, such as migration and invasion (Crosas-Molist et al., Oncogene 2016). NOX4 gene deletions are frequent in HCC patients, correlating with higher tumour grade. Here we aim to determine the cellular and molecular mechanisms regulated by NOX4 in liver cells that could explain its tumor suppressor functions.
Method: Cell models: HCC cells (PLC/PRF/5 and SNU449) were NOX4 was either silenced (ShRNA) or overexpressed. Transcriptomics, Proteomic and Metabolomic analyses. Metabolomic profile through Seahorse technology. Analysis of mitochondria structure and function. Analysis at real time of cell adhesion, proliferation and migration through xCELLigence technology.
Results: A proteomic analysis, by comparing control cells with HCC cells where NOX4 had been silenced or overexpressed, allowed the identification of metabolism as one of the highest affected processes. Silencing NOX4 in PLC/PRF/5 cells increased both the glycolysis and oxidative phosphorylation pathways, while the overexpression of NOX4 in SNU449 cells showed opposite effects. A detailed transcriptomic and metabolomic analysis indicated that NOX4 could be regulating fatty acid metabolism. We found differences in gene expression related to fatty acid transport, oxidation and de novo synthesis, as well in the amount of monoacylglycerol, diacylglycerol and carnitine intermediates, which indicate an inverse correlation between the expression of NOX4 and the cell capacity to use fatty acids. Silencing NOX4 induced changes in the amount and dynamics of the mitochondria, increase in the protein levels of complex IV and V and higher ATP levels. Preliminary results indicate that NOX4 regulates c-Myc, which could mediate the changes observed.
Conclusion: The liver tumor suppressor functions of NOX4 could be explained through its effects on HCC cell lipid metabolism.

P02-08 Regorafenib improves survival after sorafenib treatment in patients with recurrent hepatocellular carcinoma after liver transplantation, compared to best supportive care

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Background and aims: Regorafenib (REGO) has been shown to be a safe second-line treatment for hepatocellular carcinoma (HCC) recurrence after liver transplantation (LT). The aim of this study was to compare the overall survival (OS) of REGO to best supportive care (BSC) after sorafenib (SOR)-discontinuation in LT patients

Method: We conducted a retrospective, multicentre, international study, including all patients with HCC recurrence after LT who discontinued SOR. The REGO-group included patients treated with REGO after SOR-discontinuation, due to progression in tolerant patients, the control-group included all patients treated with BSC due to REGO unavailability, symptomatic progression or SOR-intolerance. The primary end-point was OS from SOR-discontinuation

Results: Included were 96 LT-patients: 32 treated with REGO after HCC progression under SOR (group-A) and 64 patients receiving BSC after SOR-discontinuation 31 SOR-tolerant in progression(group-B), 20 SOR-intolerant and 13 discontinued SOR for other reason (symptomatic progression, non-liver complications, patient's decision). The median survival for the whole cohort since SOR-start was 19.3 (13.4-25.1) months (mos). The comparative analyses were conducted between group-A and group-B, with similar clinical and demographic features [i.e. mTORi treatment 63% vs 81%

(0.11); SOR 800mg 41% vs 23% (p=0.12); SOR treatment duration 11.1 (0.7-76.7) vs 7.8 (0.9-96.3) mos (p=0.65); at SOR-discontinuation: ECOG-PS 0-1 100% vs 90% (p=0.29); tumor burden: liver only 6% vs 10%, extra-hepatic only 44% vs 45%, both 50% vs 45% (p=85); AFP: 134 (1-209,630) vs 1044 (1-88,950) ng/ml (p=0.83)]. All the 32 patients treated with REGO [treatment duration 7.0 (5.5-8.4) mos] had at least 1 adverse event (grade 3/4: fatigue in 8 and dermatological reaction in 5). Median follow-up since SOR-discontinuation was 12.3 (0.6-42.2) mos for group-A and 4.5 (0.0-22.3) for group-B (p=0.0006). At data-lock, 66% patients had died in group-A and 94% in group-B, symptomatic tumor progression being the main cause of death. The median OS from SOR-discontinuation was 14 mos (95%CI:10-18) for group-A and 4.5 mos (95%CI:24 -66) for group-B (p<0.005). The overall OS from SOR-start was 32.6 mos (95%CI:18-46) for the SOR-REGO group compared to 14.3 mos (95%CI:7-21) for the SOR-BSC sequence in the group-B (p=0.001).

Conclusion: REGO is a safe and effective second line option after SOR-progression in patients with HCC recurrence after LT

P02-09YI Nonalcoholic steatohepatitis is a risk factor for intrahepatic cholangiocarcinoma and affects its long-time outcome: Results from a multicenter international case-control study

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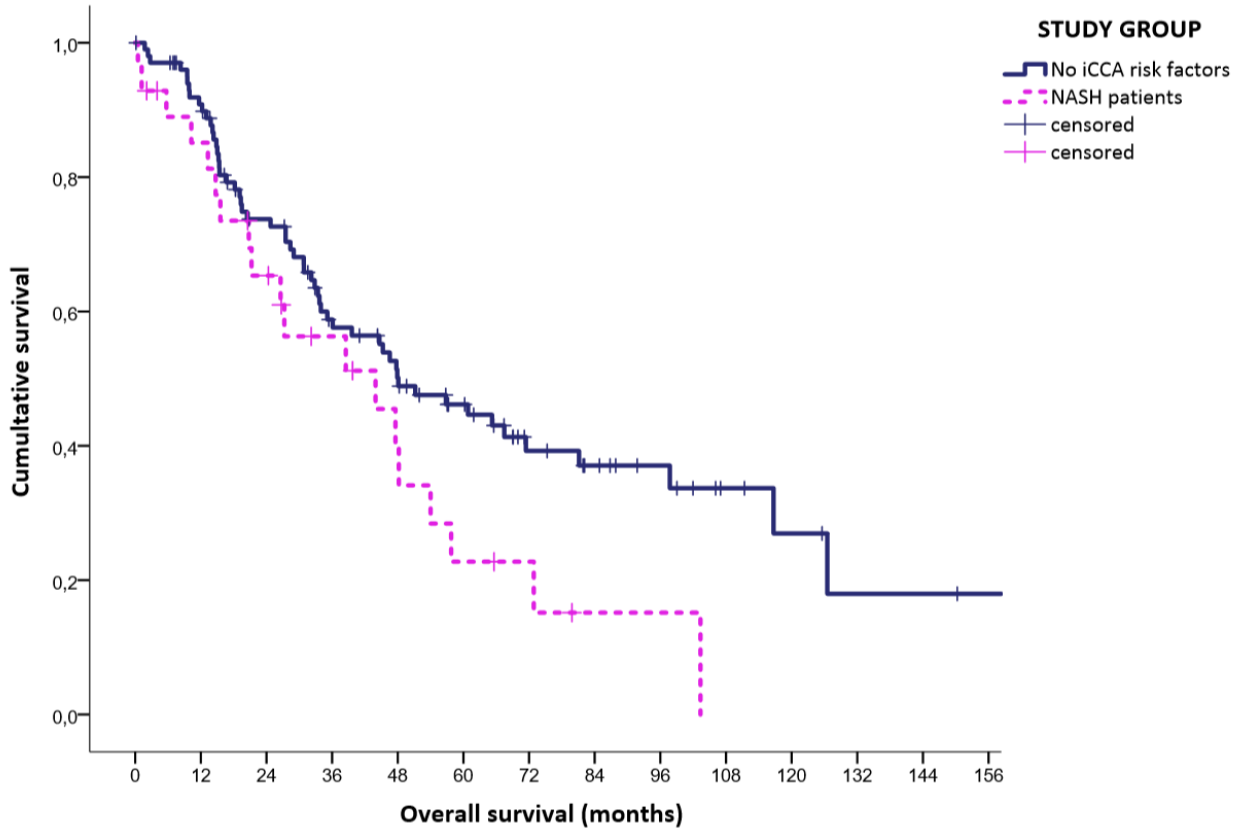
Background and aims: The prevalence of intrahepatic cholangiocarcinoma (iCCA) is rising worldwide, for reasons not fully elucidated. The current epidemics of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) might be partly responsible for this trend. Last year we reported preliminary monocentric data providing a rationale for larger studies about the pathogenic and prognostic role of NASH in iCCA. We intended to validate our results in a larger multicenter international study, verifying whether NASH is over-represented in iCCA patients (primary aim) and whether it affects iCCA outcome (secondary aim).

Method: Case-control study comparing the prevalence of histology-confirmed NASH in the peritumoral liver of resected iCCA patients (cases) and pre-explant biopsies of liver donors (controls). Controls were matched 1:1 for age and sex. In the iCCA cohort, patients with no known risk-factors for iCCA were stratified according to the presence/absence of NASH. Correlates between NASH, tumor characteristics and overall survival (OS) were subsequently explored.

Results: Between 2004 and 2017, 181 ICC patients were resected in two European centers [Bologna (Italy) and Rennes(France)]. Among the study population, 130 patients (71.8%) had no apparent risk factors for iCCA. In this group, the prevalence of NAFLD and NASH was 45.4% and 21.5%,respectively, compared to 38.5 and 6.2% in the matched liver donors (p=0.343 and p<0.001, respectively).The prevalence of NASH was similar in the two centers. Among patients without established risk factors for iCCA, main tumor size (HR 1.009 , 95%CI 1.003-1.014, p=0.003), multinodular disease (HR 1.767, 95%CI 1.092-2.861, p=0.020) and NASH (HR 2.009 , 95%CI 1.158-3.484, p=0.013) were independent predictors of the OS at the multivariate Cox regression. The median OS was 43.9 vs 48.1 months, with the two survival curves significantly diverging after the first 36 months (FIGURE). Instead, NASH was not related to an earlier recurrence of iCCA (p=0.748).

Conclusion: This multicenter study confirmed that NASH (but not NAFLD) acts as a risk factor for iCCA and affects its long-term outcome. The absence of significant differences in the time to recurrence and the late divergence of the OS curves suggest that difference in mortality is probably not attributable to a greater biological aggressiveness of NASH-related iCCA but rather to the liver- and nonliver-related complications of NASH.

Figure:


STUDY GROUP
No iCCA risk factors
NASH patients
censored
censored
Cumultative survival
1,0
0,8
0,6
0,4
0,2
0,0
0
12
24
36
48
60
72
84
96
108
120
132
144
156
Overall survival (months)

P02-10YI Albumin to gamma-glutamyltransferase and platelet to lymphocyte ratio do not predict recurrence and recurrence-free- survival after complete response to first-line HCC treatment

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Background and aims: Albumin to gamma-glutamylltranferase ratio (AGR) <0.5 and platelet to lymphocyte (PLR) > 167.7 were both reported as new independent prognostic factors of worse time-free-survival and survival in patients undergoing HCC resection. There are no data as respect to patients who achieved radiological complete response to any treatment dispensed. To this purpose we tested theses variables in patients who achieved radiological complete response after first-line treatment HCC in single tertially referral center.

Method: We retrospectively evaluated cirrhotic patients who achieved a radiological complete response (CR) to any first-line HCC treatment dispensed in a tertially referral centre between 2010 and 2018. CR was assessed by an expert rediologist one month apart the end of HCC treatment and every 4 month during follow-up, until recurrence, death or last visit,
Patient and HCC characteristics, MELD,Child-Pugh class, BCLC stage, blood tests, AGR and PLR before treatment and serum alfafetoprotein (AFP) after treatment were recorded. All the patients underwent abdominal CT scan one month apart after treatment and every 4 months during follow-up until recurrence, death or last visit. All variables with a p value < 0.1 by univariate entered a Cox multivariate analysis performed stratifing for treatment dispensed, to define independent predictive factors for recurrence, recurrent-free-survival and survival.

Results: 202 patients were enrolled: 142 (70%) male, median age 68 (range 28-86) yrs, BCLC 0-A 187 (93%), B 15 (7%), Child-Pugh A 178 (89%), B 22 (11%), monofocal HCC 151 (75%), < 3cm < 3 nodules 151 (75%).
Overall, during a median time of 22 months (95% CI 20-27), 56 (28%) patients died, 35 (17%) were lost and HCC recurred in 136 (67%): 25/44 (57%) resected, 78/111 (70%) ablative treatments and 32/47 (70%) transarterial chemoembolization. The 3yr survival, disease-free-survival and recurrence rate were 79.3% (95% 72.4-86.7), 40.4% (CI 95% 33.2-47.5) and 56.8% (95% CI 49.6-64.2), respectively.
AGR <0.5 and PRL>167.7 failed to show predictive significance even in HCC resected patients. The number of HCC nodules before starting treatment and the history of previous overt enchephalopaty were the only independent predictors of recurrence (HR1.2, 95% CI 1.0-1.5 p = 0.02, and HR 3.7 , 95% CI 1.4-9.4, p = 0.005, respectively), and of recurrent-free survival (HR 1.2, 95% CI 1.0-1.4, p = 0.05, and HR 3.1, 95% CI 1.3-7.9, p=0.01, respectively), while age > 68yrs was the only predictive factor of survival (HR 2.0, 95% CI 1.0-4.1, p 0.005).

Conclusion: AGR and PLR does not predict survival or RFS in our court of patients with radiological complete response. Number of HCC nodules and history of previous EPS are independent predictor of RFS and HCC recurrence.

P02-11 A retrospective analysis of the course of LI-RADS 3-4 hepatic lesions

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Background and aims: Hepatocellular Carcinoma (HCC) is the third leading cause of cancer-related death worldwide mostly due to it being diagnosed in late stages. Liver imaging reporting and data system (LI-RADS) is standardized criteria used to classify imaging findings of hepatic lesions in the context of surveillance for HCC in those at risk for this malignancy. LI-RADS 3 and 4 (LR-3 and LR-4) are considered indeterminate lesions and we aimed to study their natural history and the correlation of radiology interpretation to explant pathology.

Method: This is a retrospective observational study which used electronic medical records to extract data in patients who underwent liver transplant at the Hospital of the University of Pennsylvania between January 2016 to September 2019 and had HCC confirmed in explant pathology. We excluded patients with incidental HCC or without LI-RADS classification. Eligible patients were divided into 3 subgroups for statistical analysis; patients with only indeterminate lesions (LR 3 and 4), patients with only HCC lesions (LR-5), and lastly, patients with mixed type of lesions (LI-RADS 3-5).

Results: There were 114 eligible patients with a total of 99 indeterminate lesions (60 LR-3 and 39 LR-4); 87% were male, mean age was 64.6 years, and mean BMI was 27.8. Within the cohorts, 49% of cirrhosis cases were due to HCV infection, 56% had Child-Pugh score A, median (IQR) MELD-Na was 11(8-15), and median (IQR) pre-OLT peak AFP level was 8.9 (3.6-31.8). There were no differences in Child-Pugh scores or peak AFP levels among the 3 subgroups. Numbers of HCC per explant, largest HCC lesion size and cumulative size were higher in mixed lesions subgroup than the LR-5 only subgroup (p=0.007, 0.007 and 0.006 respectively). The mean size growth of LR-3 and LR-4 lesions was 2.57 and 3.8 mm, whereas their median growth rates were 0.24 and 0.39 mm/month, respectively. Higher percentages of LR-4, compared to LR-3, progressed to LR-5 (66.7% vs 33.3%) among lesions with follow-up data (p=0.004) and within shorter duration of follow-up (median 175 days in LR-4 and 196 days in LR-3). 68% LR-3 and 82% LR-4 lesions were confirmed HCC on explant pathology (p=0.09).

Conclusion: Compared to LR-3, more LR-4 lesions progressed to LR-5 (66.7% vs 33.3%), in a shorter time and with faster growth rate. High proportion of LR-3 and LR-4 lesions were confirmed HCC on explant pathology, raising a question of reliably diagnosing HCC based on radiologic criteria alone.

Figure:

Natural History	LR-3 lesions (n=60)	LR-4 lesions (n=39)
Progression per Radiologic Studies		
Progressed to LR-5	33.3%	66.7%
- Duration of progression to LR-5 (days), median (IQR)	196 (117 – 317)	175 (106 – 294)
Observations Growth		
- Size Growth (mm), mean ± S.D.	2.57 ± 4.2	3.8 ± 6.4
- Growth Rate (mm per month), median (IQR)	0.24 (0 – 0.77)	0.39 (0 – 0.95)
HCC confirmed on explant pathology (%)	68%	90%

P02-12 Early recurrence of hepatocellular carcinoma after liver transplantation can be predicted by FDG-PET and microvascular invasion at explant pathology

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Background and aims: Hepatocellular carcinoma (HCC) may recur early after liver transplantation (LT), because of pre-transplant dissemination of primary tumor cells, mainly through blood vessels. One of the main driver for recurrence is microvascular invasion, which is not always detectable by pre-operative imaging examination, as CT-scan and contrast enhanced-MRI, however the role of F-18 fluorodeoxyglucose positron emission tomography (FDG-PET) is poorly investigated in this setting. Our study aimed to identify predictors of the recurrence of HCC after LT, including FDG-PET, with particular attention to early recurrence (≤12 months) risk

Method: This is a single center, retrospective study including all consecutive patients who underwent LT for HCC as the main indication since 01/2010. Epidemiological, clinical, radiological and histological data were collected and analysed.

Results: Between 01/2010 and 07/2019 449 patients have been transplanted in our center, 182 (41%) with HCC: 158 (84%) males, median age 58 (36-71) years, 60% HCV-Ab positive, 25% MELD-score ≥15, 54% Child-Pugh stage A, median AFP at LT 9 (1-60,500) ng/mL, 29% resulted FDG-PET positive. The histopathological evaluation of the native liver revealed: 86% explanted liver with at least one active HCC nodule [median number per liver 2 (1-22)] with a median size of 32 (3-239) mm; 16% with micro-satellitosis and 27% with microvascular invasion (mVI). During a median follow-up of 42 (2-118) months, HCC recurred in 29 (16%) patients (Probability of recurrence at 1-3-5-yrs was 5-11-15%, respectively): 13 recurred within 12 months after LT (early recurrence group) and 16 >12 months. By univariate analysis, the risk-factors for early-recurrence were micro-satellitosis, mVI, G3/4, FDG-PET positive, Milan-out criteria, however only mVI and FDG-PET positivity were confirmed by multivariate analysis as independent predictors for early-recurrence (HR 0.22, p=0.019 and HR 0.11, p=0.09). The over-all survival in our cohort was 96 months (95%CI:20-89), being 49 months (95%CI:20-78) in the 13 early-recurrent patients, 64 (95%CI:39-89) in the 16 late-recurrent ones and 104 (95%CI:21-85) in non-recurrent ones.

Conclusion: The FDG-PET in the pre-LT setting and microvascular invasion at explant pathology may help to identify those patients at high risk of early HCC recurrence, deserving of aggressive surveillance or pre-emptive systemic treatment after LT.

P02-13YI A genetic risk score predicts de novo hepatocellular carcinoma in hepatitis C cirrhotic patients treated with direct-acting antivirals

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Background and aims: Several single nucleotide polymorphisms (SNPs) have been associated with hepatocellular carcinoma (HCC) in hepatitis C virus (HCV) cirrhotics, however their role in patients cured by direct-acting antivirals (DAA) is still undefined. We assessed the association between a genetic risk score (GRS) based on the combination of 4 SNPs (PNPLA3 rs738409, MBOAT7 rs641738, TM6SF2 rs5842926, GCKR rs1260326) and HCC in a cohort of DAA-treated patients.

Method: Consecutive HCV cirrhotics receiving DAA between December 2014-2016 in a single Center were genotyped. Cirrhosis was defined histologically or non-invasively (Liver stiffness measurement [LSM] ≥12 kPa). HCC was diagnosed and staged according to international recommendations. GRS score was calculated as already described.

Results: 509 patients were analyzed: median age 64 (28-87) years, 58% males, LSM 19.4 (12.0-75.0) kPa, 87% Child-Pugh score A (CPT) A. Genotypes distribution was as follows: PNPLA3 CC (46%), CG/GG (54%); MBOAT7 CC (29%), CT/TT (71%); TM6SF2 CC (91%), CT/TT (9%); GCKR CC (26%), CT/TT (74%). Median GRS score in the overall population was 0.3 (0-1.1). Patients' main clinical features were similar across SNPs genotypes. During a median follow-up of 43 (3-57) months from DAA start, de novo HCC developed in 36/452 (8%) patients, 4-year estimated cumulative probability of HCC being 9% (95% CI 7-12%). Male sex (Hazard Ratio [HR] 2.54; 95% CI 1.15-5.63; p=0.02), diabetes (HR 2.39; 95% CI 1.20-4.74; p=0.01), albumin (HR 0.35; 95% CI 0.19-0.64; p=0.001) and GRS score >0.6 (HR 2.30; 95% CI 1.03-5.11; p=0.04) were independently associated with de novo HCC. Indeed, 4-year cumulative rates of HCC resulted 6% vs. 12% in males vs. females (p=0.01); 17% vs. 7% in diabetic vs. non-diabetic (p=0.001); 21% vs. 7% in patients with albumin ≤ or >3.5 g/dl (p<0.001) and 16% vs. 7% in patients with a GRS score > or ≤0.6 (p=0.01), respectively. By combining independent risk factors for HCC, 4-year cumulative incidence resulted 20% vs. 5% in patients with or without two different risk factors, respectively (p<0.0001).

Conclusion: In a large, single-center cohort of consecutive HCV cirrhotic patients treated with DAA, a genetic risk score was independently associated with de novo HCC, together with clinical predictors (male sex, diabetes, albumin values). Combination of clinical and genetic predictors could allow better HCC risk stratification at the individual level.

P02-14YI Programmed cell death-1 gene haplotypes are associated with hepatocellular carcinoma

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Background and aims: Programmed Death-1 (PD-1) is an immune-control receptor protein on T cells which plays a role on negative regulator of self-reactivity. It was seen that PD-1 and its ligand Programmed Death Ligand-1 (PD-L1) axis has a role on immune escape of tumor cells and tumor tissues have high amount of PD-L1 levels. It was shown that there could be relationship between single nucleotide polymorphisms (SNP) in PD-1 gene (PDCD1) and susceptibility to hepatocellular carcinogenesis based on various studies. This study aimed to determine the role of three SNPs within PDCD1 gene in susceptibility to hepatocellular carcinoma (HCC) in Turkish population.

Method: We genotyped gender and age matched 137 patients with HCC and 136 controls without chronic liver disease, and history of malignancy and/or autoimmune, for PD-1.1, PD-1.5 and PD-1.6 polymorphisms with allelic discrimination analysis. The genotype, allele and haplotype frequencies were compared in HCC and control groups.

Results: No significant difference was observed in the genotype distribution of PD-1.1, PD-1.5 and PD-1.6 polymorphisms among gender and age matched HCC (M/F: 41/96; mean age: 61.4 ±11.7 years) and control group (M/F 42/94; mean age 61.4±10.1). In haplotype analysis, PD-1.1G/PD-1.5T/PD-1.6A haplotype was lower in HCC (OR=0.35, 95% CI=0.15-0.82, p=0.016) and PD-1.1G/PD-1.5C/PD-1.6A haplotype was found higher in HCC patients compared to control group (OR=50.31, 95% CI=3.03-833, p<0.0001).

Conclusion: This study was first to investigate relationship of PD-1.5 polymorphism with HCC and it was first to study relationship of PD-1.1, PD-1.5 and PD-1.6 polymorphisms with HCC among Turkish population. It was found that significant relationship between PDCD1 gene haplotypes and HCC in Turkish population. Future studies with larger sample sizes and different ethnic populations are required to validate our findings.

Table 1. Multivariate analysis of Programmed Death-1 haplotypes

					Control (n=136), n (%)	**Patient (n=137), n (%)**		
	PD.1.1	*PD.1.5*	*PD.1.6*	*Freq.*			*OR (95% CI)*	*P-value*
1	G	C	G	0.5982	166 (61)	160 (58.3)	1.00	-
2	G	T	G	0.2278	55 (20.2)	70 (25.6)	1.31 (0.85-2.01)	0.22
3	**G**	**T**	**A**	**0.0888**	**38 (14)**	**9 (3.2)**	**0.35 (0.15-0.82)**	**0.016**
4	**G**	**C**	**A**	**0.0375**	**NA**	**23 (8.4)**	**50.31 (3.03-833)**	**<0.0001**
5	A	C	A	0.0346	13 (4.8)	5 (1.8)	0.53 (0.12-2.29)	0.4

P02-15 Hepatocellular carcinoma in co-infected patients: more aggressive tumor behavior?

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Background and aims: Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer death in the world. Viral hepatitis C (HCV) and B (HBV) are the most frequent etiologies and HIV co-infection with HCV and/or HBV is present in 10% of patients. The carcinogenic effect of HIV associated with hepatitis induces a faster progression of liver disease and increases the risk of HCC, also leading to a more aggressive tumor phenotype. The aims of this study were to evaluate the survival and clinical outcomes of HCC patients co-infected with HIV + HCV/HBV, comparing them with seronegative HCC patients in the same clinical subset.

Method: This is a cross-sectional study conducted on a tertiary care hospital in Brazil. Medical records from patients diagnosed with HCC between 2007 and 2018 were gathered. We compared demographical and clinical outcomes between two groups of patients, based on their HIV serological status using logistic regression.

Results: Among a total of 267 HCC patients, 26 (9.7%) had HIV diagnosis. For HIV patients the mean age was 51 years, and 88% were male. The average time between HIV diagnosis and HCC was 13 years, and 89% were receiving antiretroviral therapy, with an undetectable viral load in 85% and a CD4+ count ranging from 76 to 417/mm3. Cirrhosis was present in 88%, being 69% HCV and 23% HBV, with Child A in 80% of patients. According to the BCLC staging system, 62% were BCLC 0-A, 15% BCLC B and 19% BCLC C. The average time between diagnosis of HCC and death was 2.5 years (1m – 12y). Although most patients were diagnosed in the early stage of the tumor, the follow-up mortality was 80%. Comparing with the seronegative patients with HCC, the seropositive group was younger by the time of diagnosis, with the mean age between 51 years for HIV positive and 61 years for HIV negative ($p< 0.0001$), with more male subjects (88% versus 71%, $p= 0.05$), but did not differ significantly on clinical scores (MELD and Child-Pugh). We also found that overall mortality was higher among HIV+ patients independently of demographical and clinical variables (80.7% vs 58.9%, adjusted OR= 5.5 (1.8-16.8), even though their time between death and diagnosis was higher (m_{HIV+}*=2,5 y,* m_{HIV-}*=0,95y,* $p< 0,0001$*).*

Conclusion: Our study demonstrated that in HIV co-infected patients, HCC appears at a younger age and presents higher mortality. Further research should be conducted to better assess the HIV role in hepatocarcinogenesis and clinical outcomes in HCC patients.

P02-16 A phase 3, randomized, double-blind, placebo-controlled study of transarterial chemoembolization combined with durvalumab (D) or durvalumab plus bevacizumab (B) therapy in patients (Pts) with locoregional hepatocellular carcinoma (HCC): EMERALD-1

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Background and aims: Since curative therapy is not always feasible and there is no standard systemic therapy, patients with intermediate-stage HCC are treated with locoregional therapy such as transarterial chemoembolization (TACE). TACE therapy achieves tumor responses, but progression and recurrence are common and often occur within 1 year.

Early evidence shows encouraging activity and durable clinical response for checkpoint inhibitors (CIs), such as durvalumab, as treatment for advanced HCC (Kelley, et al. ASCO 2017) and combined with TACE (Duffy, et al. J Hepatology, 2017). CIs combined with VEGF inhibitors (Pishvaian, et al. ESMO 2018) also show promise in advanced HCC. Taken together, combining D, VEGF inhibitors, and TACE therapies warrants evaluation in patients with locoregional HCC.

EMERALD-1 (NCT03778957) is a randomized, double-blind, placebo-controlled, multicenter Phase 3 study assessing efficacy and safety for durvalumab monotherapy when given with either drug-eluting bead (DEB)-TACE or conventional TACE (cTACE) followed by durvalumab with or without bevacizumab therapy in patients with HCC not amenable to curative therapy.

Method: 600 patients will be randomized 1:1:1 to Arm A (DEB-TACE or cTACE + durvalumab and following last TACE procedure, durvalumab + placebo), Arm B (DEB-TACE or cTACE + durvalumab followed by durvalumab + bevacizumab), or Arm C (DEB-TACE or cTACE). Durvalumab therapy will begin at least 7 days following the initial TACE procedure. Durvalumab ± bevacizumab will begin at least 14 days following the last TACE procedure.

Eligible patients must have confirmed HCC not amenable to curative therapy, have Child-Pugh score class A to B7, and an ECOG PS of 0 or 1. Patients with a history of nephrotic or nephritic syndrome, clinically significant cardiovascular disease, extrahepatic disease, or main portal vein thrombosis (Vp3/Vp4) are excluded. Patients with active (controlled) or past hepatitis virus B or C infection may be enrolled.

The primary endpoint is progression-free survival (PFS) for Arm A vs Arm C by blinded independent radiology review using RECIST v1.1. Secondary endpoints include PFS for Arm B vs Arm C, overall survival, health-related quality of life measures, and safety.

Results: N/A

Conclusion: N/A

P02-17 A novel test and treat hepatitis C micro-elimination project among underserved communities in Islamabad, Pakistan

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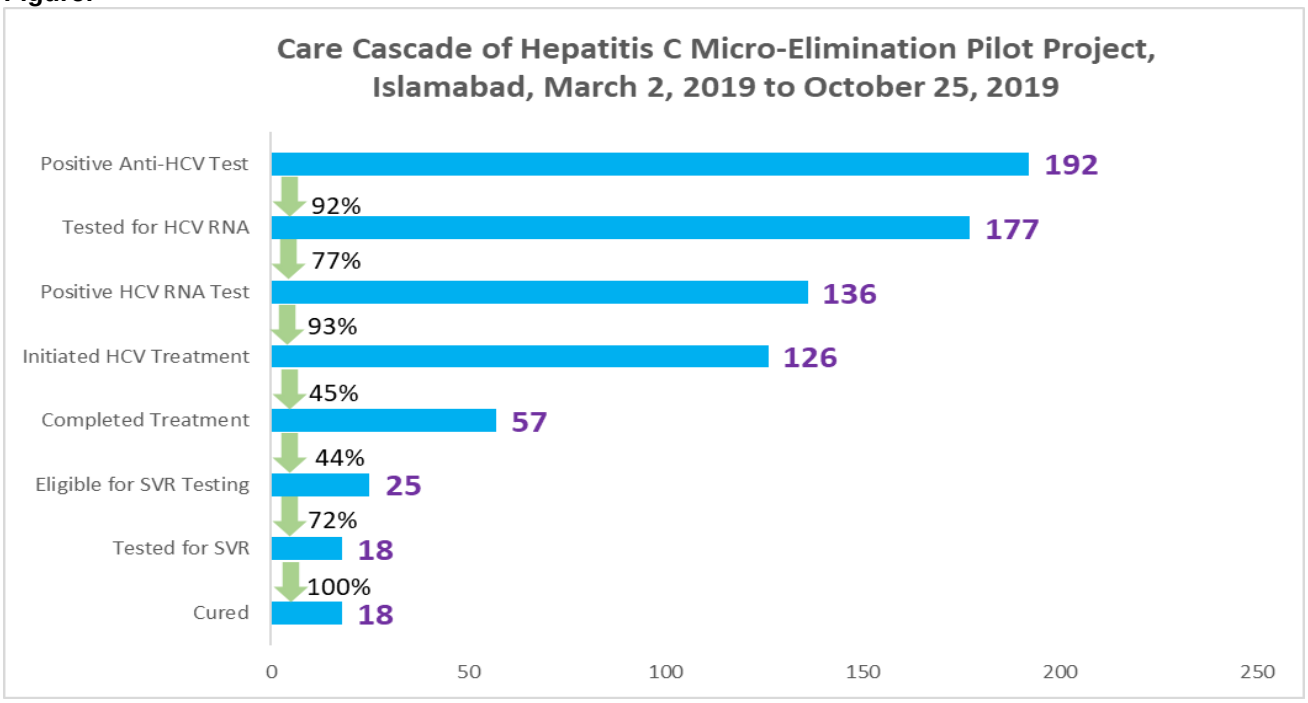
Background and aims: Pakistan has a large burden of hepatitis C virus (HCV) infection, and access to care and treatment is limited. In order to increase access for underserved populations, a same-day testing and treatment initiation model program for adults in marginalized communities (i.e *slums*) in Islamabad was launched on March 02, 2019. We describe the early results of the program.
Method: A total of 17 slums with an estimated total population of 50,000 in Islamabad have been selected by the Ministry of National Health Services, Regulations and Coordination for the project. This project includes free of charge hepatitis C testing and treatment and utilizes trained community health workers (CHWs). The CHWs visit every dwelling in the slum and offer household members aged ≥18 years screening for hepatitis C by a rapid hepatitis C antibody (anti-HCV) test. Those that test positive are referred to an established clinic for diagnosis of active HCV infection (RNA) by GeneXpert. RNA results are made available to patients within two hours. If found to be infected with HCV, additional blood is obtained and tested to calculate the AST to Platelet Ratio Index (APRI). Subjects then receive counseling, and their first 4-week supply of sofosbuvir plus daclatasvir and the first of three doses of hepatitis B vaccine during the initial clinic visit. A treatment regimen of 12 weeks for non-cirrhotic (APRI<1.5) patients is prescribed and given by the medical officer of the project. Patients with an APRI ≥1.5 are treated by a staff hepatologist. Patients are seen every 4 weeks at the clinic and given refills on their medications and queried about adverse reactions, until the end of treatment. RNA testing is conducted at 12 weeks following completion of treatment to determine cure i.e. sustained virologic response (SVR). The CHWs ensure referral and follow-up of HCV infected persons.
Results: As of October, 25 2019, a total of 5,209 participants have been screened from three *slums*, 192 (3.6%) tested positive for anti-HCV and were referred to receive testing for active infection. Of those, 177 (92%) were tested for RNA, of which 136 (77%) had active HCV infection. A total of 126 individuals (93%) had initiated treatment, of which 57 (45%) had completed treatment. To date, 18 of 25 eligible persons were assessed for SVR and all of them achieved SVR. None of the 126 individuals who initiated treatment had been previously tested or treated for hepatitis C.
Conclusion: Same day hepatitis C testing and treatment initiation is feasible among underserved communities in urban slums in Pakistan. CHWs can be effective in reaching "*hard-to-reach"* populations with limited access to health services and achieving high rates of linkage to care and adherence with treatment for hepatitis C.

Figure:


Care Cascade of Hepatitis C Micro-Elimination Pilot Project, Islamabad, March 2, 2019 to October 25, 2019
Positive Anti-HCV Test
192
92%
Tested for HCV RNA
177
77%
Positive HCV RNA Test
136
93%
Initiated HCV Treatment
126
45%
Completed Treatment
57
44%
Eligible for SVR Testing
25
72%
Tested for SVR
18
100%
Cured
18
0
50
100
150
200
250

P02-18YI Regulation of the biology of cholangiocarcinoma (CCA) cells by extracellular-signal regulated kinase 5 (ERK5)

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Background and aims: Cholangiocarcinoma (CCA) is characterized by high resistance to chemotherapy and poor prognosis. Epidermal growth factor (EGF) is involved in CCA development, and overexpression of the EGF receptor (EGFR) has been linked to tumor progression. The EGF signaling pathway may be associated with activation of extracellular signal-regulated kinase 5 (ERK5), a protein belonging to the MAPK family involved in the pathogenesis of different types of cancer. Additionally, ERK5 is implicated in cytoskeletal remodeling and cell motility. The purpose of this study was to investigate the role of ERK5 in the biology of CCA cells.
Method: Two intrahepatic human cholangiocarcinoma cell lines (HuCCT-1 and CCLP-1) and two primary human iCCA cells were used in this study. Cell growth was determined by cell counting and BrdU incorporation assay. Cell motility and invasion were assessed using modified Boyden chambers. ERK5, p-ERK5, EGFR, VEGF and Angiopoietin 1 were investigated by Western blotting. Silencing of cells was performed by gene silencing with shRNA. XMD8-92 and AX15836 were used to inhibit ERK5 activity.
Results: ERK5 was upregulated in all CCA cells examined and phosphorylation of ERK5 was increased in cells exposed to EGF. Growth of CCA cells in serum-containing medium was decreased after exposure to 10μM XMD8-92. In addition, migration and invasion induced by EGF were significantly reduced by both XMD8-92 and AX15836 (2μM). Similar results were obtained in ERK5-silenced cells exposed to EGF, when compared to treated with non-targeting (NT) shRNAs. In addition, in ERK5 silenced cells, expression of VEGF and angiopoietin 1 was reduced compared to NT cells. Of note, conditioned medium (CM) obtained from HuCCT-1 cells induced an increase in migration of both human hepatic stellate cells (HSC) and THP-1 monocytes, an effect reduced when conditioned medium from ERK5-silenced cells was used. Furthermore, the inhibitory effects of metformin on cell growth were more evident in ERK5-silenced cells.
Conclusion: In cholangiocarcinoma cells, ERK5 activity regulates cell growth and motility, release of angiogenic factors and drug resistance.

P02-19 A blood-based DNA methylation test for early detection of hepatocellular carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) is among the leading causes of cancer-related mortality worldwide and given its asymptomatic nature in the early stages of the disease, the majority of HCC cases are detected in advanced stages. Thus, early diagnosis of HCC in patients with known risk factors remains as a critical challenge to improve patient's survival rates and clinical outcomes. Here, we report a blood-based DNA methylation test which can be leveraged to detect HCC at early stages from tumor-derived circulating cell-free DNA (cfDNA) biomarkers.

Method: The blood-based test uses a DNA methylation-based panel that sensitively detects cancer-specific DNA methylation patters of cfDNA isolated from blood samples. Plasma samples were collected from 205 subjects, including: 35 subjects diagnosed with hepatocellular carcinoma (Stage I to IV), 51 healthy subjects with no history of liver disease, 86 subjects diagnosed with benign diseases (cirrhosis, fatty liver disease, and/or hepatitis B or C virus infection) and 33 subjects with a diagnosis of a cancer other than liver cancer (breast, colon, lung, ovarian, melanoma). Cell-free DNA was extracted from samples, bisulfite converted, and DNA methylation was quantified at target sites.

Results: By using a pre-established clinical cutoff 28 of the 35 samples drawn from subjects with hepatocellular carcinoma were correctly identified for an overall sensitivity of 80% (95%CI: 67-93%) and an overall specificity of 86% (95%CI: 80-92%) for subjects not diagnosed with cancer. The specificity of the test for healthy subjects and subjects diagnosed with a benign liver condition were separately calculated to be 88% and 85%, respectively.

Conclusion: These data support the potential of this blood-based HCC-specific methylation panel as an early detection test for hepatocellular carcinoma and improve patient outcomes.

P02-20 miRNA profiling of biliary intraepithelial neoplasia reveals step-wise tumorigenesis in distal cholangiocarcinoma via the miR-451a/ATF2 axis

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Background and aims:
Biliary intraepithelial neoplasia (BilIN) is the common precursor lesion of distal cholangiocarcinoma (dCCA). However, the sequence leading from a single epithelial layer in normal biliary epithelia to intraepithelial precursor lesions and finally to the invasive tumor is poorly understood. Here, we aimed to elucidate key miRNAs involved in this step-wise process and to identify their mRNA target genes in cholangiocarcinogenesis.

Method:
As basis of our study, we conducted Laser Capture Microdissection of FFPE tissue sections of 12 patients with dCCA. For each patient we isolated three matched sample sets, including non-neoplastic biliary epithelia, high-grade BilIN, and invasive dCCA, thereby representing different stages of distal cholangiocarcinogenesis. This resulted in a total of 36 samples. Total RNA was extracted and the expression of ~800 miRNAs was assessed using the Nanostring® technology. Significantly deregulated miRNAs were validated by quantitative RT-PCR. Target genes were identified using miRWalk2.0 and validated by qRT-PCR and Western blot.

Results:
We identified 49 miRNAs with a significantly altered expression from non-neoplastic biliary epithelia to dCCA of which 23 showed a gradual up- or downregulation. Clustering analyses separated the samples into three groups: non-neoplastic tissue, high-grade BilIN, and invasive dCCA. Next, we selected candidate miRNAs by fold change expression of invasive dCCA versus high-grade BilIN versus non-neoplastic biliary epithelia to reflect the stepwise tumorigenic process. Subsequently, the following top 4 miRNAs were identified: miR-23a-3p (2.1-fold up), miR-10a-5p (9.7-fold up), miR-144-3p (6.3-fold down) and miR-451a (10.9-fold down). The expression of all candidate miRNAs was successfully validated by RT-qPCR. The online tool miRWalk2.0 predicted the metastasis-related genes ATF2 and ADAM10 to be direct miR-451a targets. In vitro experiments confirmed that miR-451a expression leads to a downregulation of ATF2 and ADAM10 in dCCA cell lines. Functional experiments showed a repression of cell migration by overexpression of miR-451a and ATF2 inhibition by pooled siRNAs mimicked this effect.

Conclusion:
Thus, our data support the concept of high-grade BilIN as a direct precursor of invasive dCCA. In addition, we identified potentially tumor suppressive and oncogenic candidate miRNAs. miR-451a stood out as a potential tumor suppressor inhibiting cell migration by targeting ATF2.

P02-21YI Plasma circulating cell-free DNA integrity is a risk factor of denovo hepatitis C related hepatocellular carcinoma treated or not with direct acting antiviral therapy

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Background and aims: The clinical value of plasma circulating cell-free DNA (cfDNA) integrity as diagnostic biomarker in the patients with HCV related hepatocellular carcinoma (HCC) was investigated and correlated with the commonly used marker alpha fetoprotein (AFP).
Method: This case control study was conducted on 80 patients with HCV genotype 4-related liver cirrhosis during the period from January 2018 to September 2018. They were divided into two groups: **Group 1:** 40 patients with HCC divided into: **Group 1a:** 20 patients with HCC who were naïve to antiviral therapy. **Group 1b:** 20 patients with HCC who previously received direct acting antivirals (DAAs) and achieved sustained virologic response. Plasma cf-DNA integrity using *ALU 115* and *ALU 247* sequences was assessed using SYBR green based Real-Time polymerase chain reaction (PCR). Cf-DNA integrity was calculated as the ratio of Q247/Q115, where Q115 and Q247 are the ALU-qPCR results obtained with the ALU 115 and ALU 247, respectively
Results: There was no significant difference among the 2 groups of patients with HCC regarding their baseline demographic or laboratory features. patients with HCC had significantly lower plasma cf-DNA integrity than those with liver cirrhosis without HCC. There was no significant difference in the circulating plasma DNA integrity between HCC patients who previously received DAAs and those who did not. There was a significant negative correlation between AFP and plasma cf-DNA integrity ($r = -0.54$, $P < 0.001$). There was a negative correlation between the number of hepatic focal lesions and the plasma cf-DNA integrity (r value = - 0.372, P value < 0.005). ROC curve revealed an area under the curve (AUC) for detection of HCC by cf-DNA integrity and AFP were 0.965 and 0.886 respectively. Adding cf-DNA integrity to AFP as an adjuvant of diagnosis, improved the sensitivity of the two markers together compared to AFP alone from 81.6% to 94.7%, positive predictive value from 93.4% to 94.7%, negative predictive value from 84.4% to 94.9% and accuracy from 88.4% to 94.8%.
Conclusion: Plasma cf-DNA integrity can be used as a potential early marker for HCC among the HCV patients.

Figure:

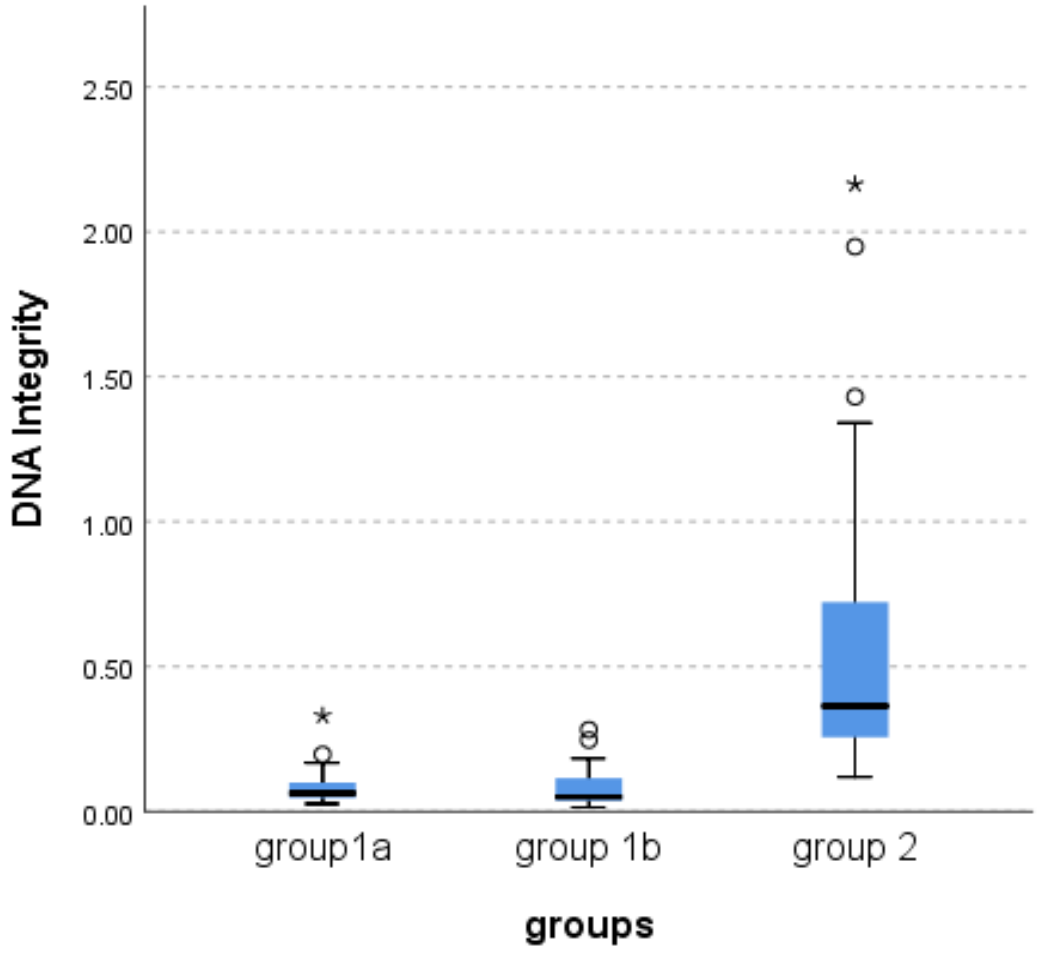

Figure (1): Box and whisker plot of DNA integrity index among the three groups

P02-22 The role of stereotactic body radiation therapy in the management of hepatocellular carcinoma: Outcome and toxicity from a single institution experience

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Background and aims: *Standard management of Hepatocellular Carcinoma (HCC) includes surgery , transcatheter arterial chemoembolization (TACE), radiofrequency ablation (RFA), percutaneous ethanol injection (PEI), or systemic therapies. Stereotactic body radiotherapy (SBRT) recently emerged as an option in HCC patients ineligible for standard local therapies or following incomplete response after primary treatment. The aim of this study is to evaluate outcome and predictors of response to treatment in a retrospective HCC cohort treated with SBRT.*

Method: Clinical and treatment-related data from HCC patients treated with SBRT at our institution between were retrospectively reviewed. Biological Effective Dose (BED) was calculated to compare different dose regiments. Local control (LC), Progresion-Free Survival (PFS) and Overall Survival (OS) at 1 and 2 years were calculated. Toxicity was scored using the Common Terminology Criteria for Adverse Events (CTCAE) v.4.03.

Results: One-hundred-seventeen HCC patients were included, classed according to Barcellona staging (BCLC) system in A, B and C in respectively 39 (33%), 64 (55%) and 14 (12%) cases . Median age was 75 (range 45-88) years. SBRT was delivered to a median dose of 54 (range 30-78) Gy in 6 (range 3-10) fractions, corresponding to a median BED of 120 (range 56-263) Gy^{10}. Median cumulative gross tumor volume (GTV) size was 40 (range 8-116) cc. One-year LC rate was 82% (CI95%:72-89). Among the examined variable (age, aetiology, BCLC, thrombosis, multiple foci, prior treatment, cumulative GTV size, BED) only BED ≥120 Gy^{10} was correlated to improved LC (median not reached, p= 0.0046). One-year PFS was 41% (CI95%:32-51) with intrahepatic failure as the dominant pattern of primary recurrence in 45% (n=57) of patients: no variable was associated to a poorer PFS. One-year OS was 82% (CI95%:70-90): only prior surgery was correlated to improved survival (median 29 versus 17 months, p=0.036). Overall toxicity was reported in 15% (n=18) of patients.

Conclusion: SBRT is a safe procedure and provides high 1-year LC in HCC patients, in particular if a dose regimen delivering a BED ≥120 Gy^{10} is applied. Despite excellent local control, out-of-field progression remains of concern. SBRT following prior surgical resection is correlated to improved survival.

P02-23 Development of personalised human immunocompetent ex vivo models of primary and secondary liver cancers using precision cut tissue slice technology

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Background and aims: Experimental models of liver cancers lack the complex interactions between the immune system and tumour, they also fail to capture inter-individual variability, 3D tissue architecture, cellular heterogeneity and tumour specific immune landscape. This has hindered the understanding of the pathogenesis of disease and development of personalised treatment approaches. We aimed to develop ex-vivo human immunocompetent models of primary (hepatocellular carcinoma; HCC) and secondary (neuroendocrine liver metastasis; LM-NEN) liver cancers using precision cut tissue slice (PCTS) technology from surgical waste for discard or explanted tissue.

Method: To date, 6 HCC and 5 LM-NEN tissue samples have successfully been collected and sliced using previously established protocols. PCTS were cultured ex-vivo for 8-15 days in i) 95% O2, ii) atmospheric O2, iii) atmospheric O2 + microfluidic organ-on-a-chip system (CNBio). Each day the viability of slices was assessed by measuring apoptotic vs non-apoptotic cell death (cytokeratin 18), lactate dehydrogenase release, ATP content and histological analysis. Immunofluorescence was used to quantify proliferative capacity (Ki67) and neuroendocrine differentiation (chromogranin A (LM-NEN)). Metabolic capacity was examined by measuring adenylate energy charge using HPLC. Innate and adaptive immune components of the slices were interrogated using PCR microarray analysis.

Results: Histologically, the characteristic tissue architecture including tumour morphology, stroma and immune infiltration is maintained over the culture period and tissue viability markers remained stable. HCC slices remained viable for up to 8 days and preferred a normoxic environment whereas LM-NEN could be maintained for 15 days when cultured in high oxygen conditions. This was confirmed by consistently low levels of apoptotic cell death (<5%) over duration of culture. Proliferative capacity remained constant and distinctive immunological features associated with an immune infiltrated ('hot') or immune deserted ('cold') tumour micro-environment were preserved in the slices.

Conclusion: We have successfully developed a human personalised ex-vivo model of primary and secondary liver cancers that retain the structural, metabolic and immunological signatures observed *in vivo*. This model is ideal to understand the immunopathogenesis of liver cancers and could potentially be used to develop a personalised medicine approach for patients to determine best treatments for residual disease post-surgery.

P03-01YI Tumour burden score: A useful tool to predict immune-related hepatotoxicity during immunotherapy in HCC

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Background and aims: Nearly 10% of patients treated with immune checkpoint inhibitors (ICI) for unresectable/advanced hepatocellular carcinoma (HCC) experience G3-G4 hepatic immune-related adverse events (HIRAEs). While the risk factors for such adverse events are largely unknown, we assessed the role of tumor burden as a determinant for development of HIRAEs.

Method: We selected 36 patients with HCC treated with a monoclonal antibody (mAb) targeting the programmed cell death receptor-1 or its ligand (PD-1/PD-L1) as single agent (16 patients, 44%) or in combination with a mAb against the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) (20 patients, 56%). The pretreatment Tumor Burden Score (TBS) takes into account both the total number of liver nodules (a) and the maximum diameter (b) according to the following formula $TBS^2 = a^2 + b^2$. HIRAEs were categorized according to the Common Terminology Criteria for Adverse Events (v. 5.0).

Results: 18 patients (50%) developed any grade HIRAEs, of whom 5 (18%) developed G3-G4 HIRAEs. No G5 toxicity was registered. No patient permanently discontinued treatment because of HIRAEs. Patients who developed any grade HIRAEs had significantly higher mean values of TBS compared to patients with no HIRAEs (13.4 [95% CI 8.3 - 18.5] vs 7.3 [95% CI 3.7 - 11.1], p = 0.048). In particular, patients with a TBS of 10 or more had a significantly higher risk of developing any grade HIRAEs compared to patients with TBS < 10 (Odds ratio = 5, p = 0.041). The development of G3-G4 HIRAEs was not associated to a significantly higher TBS compared to patients with G0-G2 HIRAEs (12.0 [95% CI 8.23 - 32.2] vs 10.1 [95% CI 6.82 - 13.32], p = 0.70). Median overall survival did not significantly differ between patients with TBS < 10 and patients with TBS ≥ 10 (7.9 months vs 6.3 months, p = 0.60).

Conclusion: In HCC patients treated with ICI, hepatic tumor burden could be a risk factor for the development of any grade HIRAEs. TBS is a useful tool to measure hepatic tumor burden and it could be used to predict the risk of HIRAEs. The role of TBS in predicting HIRAEs needs to be confirmed and validated in larger cohorts of HCC patients.

P03-02YI PIVKA-II is a useful biomarker for hepatocellular carcinoma in caucasian HCV cirrhotic patients treated with direct-acting antivirals

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Background and aims: Prothrombin induced by vitamin K absence-II (PIVKA-II) has been shown a useful marker for hepatocellular carcinoma (HCC), however its performance in HCV Caucasian cirrhotics treated with direct-acting antivirals (DAA) is still unknown. We evaluated PIVKA-II and AFP values in DAA-treated HCV cirrhotics.

Method: Consecutive HCV cirrhotics receiving DAA in a single center were tested for PIVKA-II and AFP by a fully automated chemiluminescent enzyme immunoassays on Lumipulse G1200 analyzer (Fujirebio, Japan) at DAA start (baseline), end of treatment (EOT), post-treatment week 12 (PTW12) and eventually at HCC diagnosis. Patients underwent regular 6-months HCC surveillance with abdominal ultrasound (US).

Results: Overall, 692 sera from 214 patients were tested, median age 63 (28-87) years, 60% males, 19% diabetics, 87% Child-Pugh score A, LSM 17.5 (12.0-75.0) kPa. A sustained viral response (SVR) was achieved by 210 (98%) patients. Median AFP levels significantly decreased from 15 (1-537) ng/mL at baseline to 8 (1-347) at EOT and 6 (2-48) at PTW12 ($p<0.0001$), while PIVKA-II values did not significantly change, being 37 (12-520) AU/L at baseline, 37 (14-867) at EOT, and 40 (20-1,192) AU/L at PTW12 ($p=0.10$). After a median follow-up of 42 (3-57) months, 41 patients developed HCC, with a 4-year cumulative incidence of 9% (95% CI 7-12%). At diagnosis, median HCC size was 20 (10-30) mm, single nodule in 68%, BCLC 0-A in 85%, median AFP 7 (2-12,868) ng/mL and PIVKA-II 55 (22-15,283) AU/L. By applying the PIVKA-II >40 AU/L and AFP >7 ng/mL cutoffs, 33 (80%) HCC tested positive for at least one marker [28 (68%) PIVKA-positive, 19 (46%) AFP-positive, 8 (19%) negative for both markers]. In terms of kinetics before HCC onset, at least one marker increased above the cutoff in 10 patients following EOT timepoint, whereas median time to HCC diagnosis by US-based surveillance was 39 (1-52) months after EOT. The diagnostic accuracy for HCC resulted 68% for AFP > 7 ng/mL, 73% for PIVKA-II > 40 AU/mL, reaching 95% when combining both markers. The 4-year probability of HCC resulted 41% vs. 13% in patients with PIVKA-II > or ≤40 AU/L at EOT ($p<0.00001$), 31% vs. 20% in patients with AFP > or ≤7 ng/mL ($p=0.09$) and 45% vs. 20% in patients with or without both markers increased ($p=0.002$).

Conclusion: In HCV cirrhotic patients treated with DAA, PIVKA-II levels, in combination with AFP, are associated with HCC development.

P03-03YI External validation of the Toronto hepatocellular carcinoma risk index in Turkish cirrhotic patients

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Background and aims: Toronto Hepatocellular Carcinoma Risk Index (THRI) is developed to stratify cirrhotic patients according to 10-year Hepatocellular Carcinoma (HCC) risk. We aimed to validate the performance of THRI in a large Turkish cohort.

Method: We retrospectively reviewed the database of 1287 cirrhotic patients followed-up in a 10-year period (February 2008-January 2018). All patients were stratified into three groups based on the THRI score as follows; low-risk:<120, intermediate risk:120-240 and high risk:>240. Area under the curve (AUC) and optimal cut-off value of THRI was obtained from receiver operator curve (ROC). To reveal the parameters related with HCC development, logistic regression analysis was conducted. The cumulative incidences of HCC were calculated using Kaplan-Meier method, and the curves were compared using the log-rank test.

Results: Out of 403 enrolled patients, 57 developed HCC. The median THRI value was higher in HCC (+) group comparing to HCC (-) group (267 (70-366) vs 224 (36-366), p<0.001). Out of 57 detected HCCs, 45 (78.9%) were high risk, 11 (19.3%) were intermediate risk and only one (1.8%) was low risk at the entry. The AUC of the THRI to predict HCC was 0.750(%95 CI 0.683-0.817, p<0.001). The optimal cut-off value of THRI was 239.5, giving a sensitivity of 78.9% and specificity of 62.7%. As a result, THRI remained to be the only significant parameter that has an affect on HCC development (adjusted-OR:1.016 (95%CI 1.007-1.024), p<0.001).

Conclusion: The present study validated the performance of THRI in cirrhotic patients to predict HCC risk, which can be considered as a tool for personalized surveillance.

Figure:

Table 1. Parameters associated with HCC development

	Univariate			Multivariate		
	p value	OR	95%CI	p value	OR	95%CI
THRI	0.000*	1.015	1.009-1.020	0.000*	1.016	1.007-1.024
AST	0.190	1.003	0.999-1.007	0.879	0.998	0.969-1.027
ALT	0.555	1.002	0.996-1.007	0.858	0.998	0.973-1.023
ALB	0.007*	0.561	0.370-0.851	0.101	0.376	0.117-1.210
INR	0.107	2.939	0.793-10.892	0.160	15.247	0.342-679.386
T.bil	0.837	1.029	0.783-1.352	0.326	0.700	0.343-1.428
Cre	0.538	0.688	0.209-2.260	0.353	0.363	0.43-3.081
AFP	0.081*	1.045	0.995-1.098	0.669	1.028	0.905-1.169
FIB-4	0.002*	1.104	1.037-1.177	0.979	0.997	0.771-1.288
APRI	0.047*	1.132	1.001-1.281	0.827	1.085	0.523-2.247
BMI	0.327	1.033	0.968-1.103	0.196	1.053	0.973-1.140
MELD	0.225	1.085	0.951-1.237	0.144	0.746	0.504-1.105
CPS	0.283	1.117	0.913-1.367	0.932	0.973	0.520-1.822

AFP: Alpha-feto protein, ALT: Alanine transaminase, Alb: Albumin, AST: Aspartate transaminase, APRI: AST to Platelet Ratio Index, BMI: Body-mass index, Cre: Creatinine, CPS: Child-Pugh Score, FIB-4: Fibrosis-4 Score, MELD: Model for end-stage liver disease, INR: International normalized ratio, T.bil: Total bilirubin THRI: Toronto Hepatocellular carcinoma risk index

P03-04YI Deletion of erk5 protein kinase in myeloid cells impairs liver regeneration in mice

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Background and aims: During hepatic injury and repair, myeloid cell population plays a crucial role in the control of local tissue inflammation and regenerative response to maintain tissue architecture. ERK5 is a member of MAPKs family and it is involved in the modulation of a wide variety of cellular responses, including cell survival, differentiation and proliferation. Besides, ERK5 modulates inflammation in endothelial cells and monocytes and the differentiation of monocytes to functioning macrophages was found to be dependent on ERK5 signaling. ERK5 has emerged as a crucial component of oncogenic signaling and we demonstrated that ERK5 pathway is critical for the development and growth of hepatocellular carcinoma and we generated a murine model in which the ERK5 gene was selectively ablated in the myeloid lineage (*LysERK5KO*). This study aimed to investigate the phenotypic response of *LysERK5KO* mice subjected to partial hepatectomy (PH) in order to characterize the function of ERK5 in hepatic regenerative response after PH.
Method: The *LysERK5KO* animals were generated crossing the *ERK5 floxed* mice (Control mice) with mice expressing Cre-recombinase under the control of M lysozyme promoter (*LysMCre*). Animals of 16-24 weeks of age were subjected to standard PH. Serum ALT and AST were measured using standard assays. Gene expression was assayed by RT-PCR.
Results: To investigated the effect of myeloid-specific ERK5 deletion on liver regeneration, control and *LysERK5KO* mice underwent PH and were euthanized at 8, 24, 48 and 168 hours. Liver-to body weight ratio showed that liver recovery was slightly slowed down at 24 and 48 h after PH in *LysERK5KO* mice. Measurement of PCNA showed that this phenotype was accompanied by mild reduction hepatocyte proliferation. Interestingly, ALT and AST levels were strongly increased in *LysERK5KO* mice after PH and histological analysis showed a higher degree of hepatic tissue damage in *LysERK5KO* mice. At this point, we decided to analyze hepatic macrophage polarization in our experimental groups because it has been demonstrated that enhanced M2 macrophage polarization in the liver is associated with improved liver repair and regeneration whereas M1 polarization triggers tissue damage. The measurements of mRNA levels of M1/M2 markers showed a reduced expression of M2 markers (ARG1, MRC2) and a parallel increase of M1 markers (CCL2, IL1β) in *LysERK5KO* group (48 h after PH) compared to *ERK5 floxed* mice.
Conclusion: The results obtained indicate that ERK5 could contribute to liver regeneration by modulating macrophage plasticity. In particular myeloid-specific ablation of ERK5 causes a severe liver damage and this phenotype seems to be associated with enhanced M1 macrophage polarization in the liver. Further studies are needed to characterize ERK5 as critical regulator of macrophage polarization and innate immune system during liver regeneration process.

P03-05YI cfDNA in hepatocellular carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) is the 6th most common cancer worldwide (WHO,2019) and accounts for the 4th most cancer-related deaths (WHO,2019). As a growing health burden, additional biomarkers are needed to aid early diagnosis, prognosis, and to support treatment plans for the management of HCC. Studies have supported the use of cell-free DNA (cfDNA) (Madsen et al., 2019), released primarily from apoptotic and necrotic cells, as a novel non-invasive biomarker. While clinical utility still needs validation, the aim of this study was to explore cfDNA in correlation with clinical outcomes and sequence plasma cfDNA for mutational analysis, as well as investigate how transarterial chemoembolization (TACE), a type of anti-angiogenic targeted chemotherapy, affects cfDNA levels.

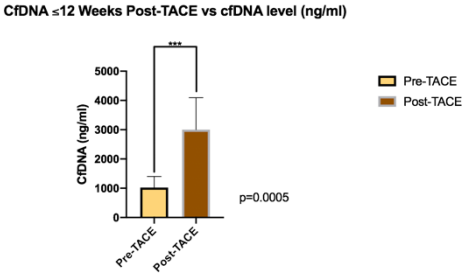
Method: Plasma was isolated from patients with HCC (n=78) from three centres (UK, Germany, Italy). cfDNA was extracted using a silica-based column method, vacuum manifold (QIAGEN), and quantified using Qubit (Thermo Fisher). Extracted samples were sent for next generation sequencing, pending results. cfDNA levels were analysed pre- and post-TACE, as well analysed in association with clinical outcomes.

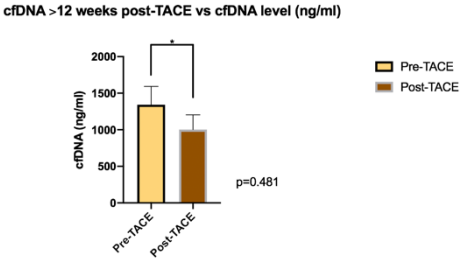
Results: Plasma cfDNA post-TACE was analysed against weeks elapsed between sample collection after the procedure (n=56). An increase in plasma cfDNA was detected post-TACE in samples collected <12 weeks after the procedure (*1023 ng/ml vs 3004 ng/ml, p= 0,0005*). Conversely, plasma cfDNA decreased in samples collected >12 weeks post-TACE (*1342 ng/ml vs 1002 ng/ml, p=0.481*). The presence of portal vein thrombosis (PVT) showed a trend towards higher plasma cfDNA, while cirrhosis appeared to have no effect. BCLC stage A showed a trend towards lower plasma cfDNA pre-TACE compared to stage B and C. Furthermore, there appeared to be a trend showing lower cfDNA in patients who presented with one tumour versus multiple.

Conclusion: There is a correlation between BCLC stage, PVT, and number of tumours with pre-TACE plasma cfDNA levels, where lower tumour burden correlates with lower plasma cfDNA. Furthermore, an increase in plasma cfDNA <12 weeks post-TACE suggests DNA release by apoptotic and necrotic cells targeted by the procedure. However, after 12 weeks, a decrease in plasma cfDNA below baseline suggests a lower tumour burden. Mature data will be presented with the entire data set and sequencing data. cfDNA has the potential to support clinical decision making in HCC.

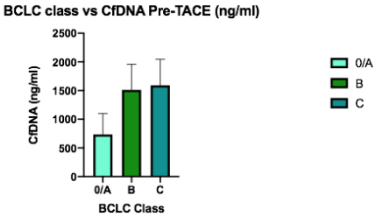
Figure:

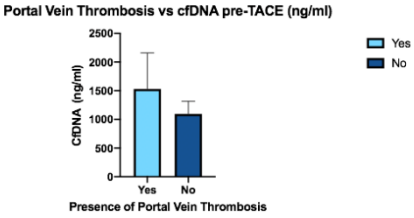
1. TACE and cfDNA

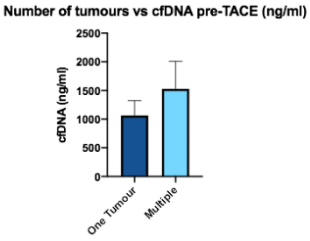


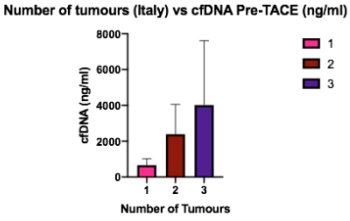


2. Clinical Outcomes and cfDNA









P03-06YI The impact of postoperative ascites on survival after hepatectomy for hepatocellular carcinoma: A multicentric nation-based analysis

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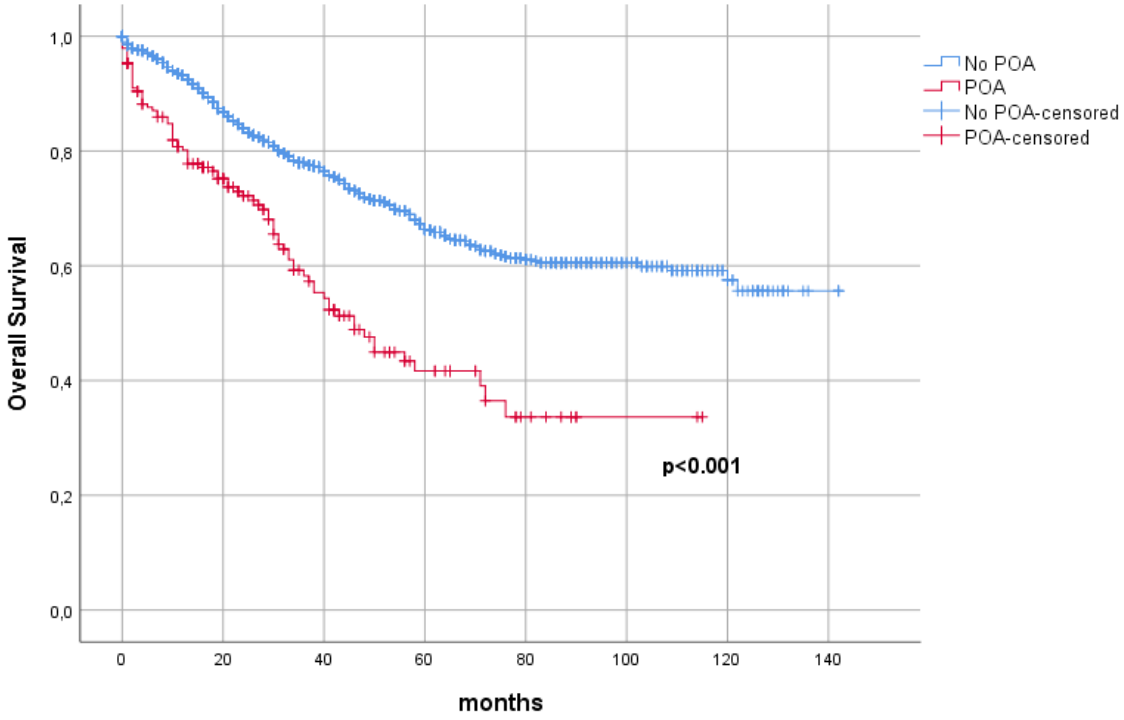
Background and aims: The occurrence of post-operative ascites (POA) is a common but unreported complication after hepatectomy for hepatocellular carcinoma (HCC). The aim of our study was to investigate the frequency of ascites, its impact on overall survival (OS) and disease-free-survival (DFS), and define which factors might be associated with its occurrence.

Method: Data were collected from 23 centers participating to the Italian Surgical HCC Register (HE.RC.O.LE.S. Group). Postoperative ascites was defined as ≥500ml of ascites In the drainage after surgery for at least 3 consecutive days. Survival was estimated by Kaplan-Meier, and risk-adjustment analysis was performed by Cox Regression.

Results: Among 1849 patients resected for HCC between 2007 and 2018, 1,656 (89.5%) patients did not experienced POA while 193 (10.4%) experienced this complication. Presence of cirrhosis (OR= 1.995; 95%CI=1.12-3.43; p=0.006) and varices (OR=2.323; 95%CI=1.32-4.07; p:0.002) were predictors of ascites, while laparoscopic surgery was found be protective (OR=0.230; 95%CI=0.12-041; p<0.001). Ninety-day mortality was higher in the POA group (8.8% vs 1.7% in no-POA group, p<0.001). Median OS for those patients who did not have POA was not reached, while it was 46 months (95%CI=35.66-56.33) for those who developed ascites (p<0.001). After risk-adjustment for confounders, POA independently increased the risk of mortality (HR=1.465; 95%CI=1.01-2.10; p:0.040) together with presence of microvascular invasion (HR=1.867; 95%CI=1.30-2.67; p:0.001). Relapse risk after surgery was not independently predicted by the occurrence of POA.

Conclusion: The occurrence of POA was a relative frequent complication after hepatectomy for HCC. However, its occurrence was significantly associated with worse survival. More efforts should be done to limit such event.

Figure:



Overall survival and comparison of the group with post-operative ascites (POA) versus the group who did not experienced the postoperative event.

P03-07YI Biochemical and clinical evidence of hepatocellular carcinoma among poorly viral suppressed ART initiators in Southwestern Nigeria

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Background and aims: Latest findings documented that HIV viremia with poor viral suppression and low CD4 are associated with increased risk of liver cancer in this era of save ART since viral suppression is the hallmark of HIV treatment (Torgersen et al, 2019). Presently there are flip reviews and no published data to correlates long viral suppression with liver cancer risk among HIV Nigerian populace on ART. Hence the aim of this study was to evaluate the clinical and biochemical evidence of hepatoma carcinoma(HCC) among early and poorly suppressed viral ART initiators in a Government treatment hub in South Western part of Nigeria.
Method: Six months after initiation of ART, 90 ART initiators (with no evidence of Hepatitis B and C) from the treatment hub was followed up and grouped as follows after ethical and informed consent was sought. <20copies/ml (Early suppressed, n=45), 1000-10,000copies/ml (Poorly suppressed, n=45) and positive control group (n=35) of confirmed cases of primary liver cancer with no evidence of HIV viremia. Clinical and biochemical investigations including oxidative stress markers were evaluated.
Results: Clinical features generally observed among ART intiators are hepatomegaly 71/90(78.9%), Jaundice 73/90(81.1%),Pedal Edema 48/90(53.3%).Poorly suppressed group shows 3.5× serum albumin lower with seven, five, four fold increased similar to positive controls for AST/ALT ratio, Bilirubin, ALP of the diagnostic reference range. Alpha feto protein in poorly suppressed and positive control was statistically superior to early suppressed group (P=.03 and P=.01). In addition 73% had prolong prothrombin time and was associated with increase risk of developing HCC in univariate (hazard ratio [HR]=3.8; 95%l[CI]=I.3-12.9;p=.04) and multivariable analysis (HR=4.6; 95% CI =1.4-14.8; P=.02) and low platelet counts (HR=2.3; 95% CI= 1.7-2.9; P<.01) were significantly associated with increased risk of developing HCC in multivariable analysis. Oxidative stress was in significant higher proportion in poorly suppressed than the early suppressed group (38.9%[95%CI,16.5%-61.5% Vs 5.6%[95%CI,0-15%; P= .04) on total antioxidant capacity reference value put at 1.117±0.007.

Conclusion: Individuals with higher viral copies show a stronger association with hepatocellular carcinoma risk. The institution of hyperbilirubinaemia, hypoalbuminaemia, thrombocytopaenia, prolong prothrombin time, raised alpha feto-protein and liver enzymes coupled with severe oxidative stress implicated among the poorly suppressed ART initiators was consistent with liver cancer manifestation. The above possibly suggest the presence of either pro-oncogenic HIV encoded proteins that are associated with HIV viremia or drug (ART) induced hepatoxicity which are contributing factors to the progression of chronic liver diseases and hepatocarcinogenesis.

P03-08 Optimisation and qualification of tumour mutational burden (TMB) by targeted next-generation sequencing (tNGS) as a clinically applicable biomarker in hepatocellular carcinoma (HCC)

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Background and aims: Higher levels of somatic non-synonymous mutations associate with improved response and survival following immune-checkpoint inhibitors. In this pilot study, we aimed to optimise tNGS as a method to provide a reliable estimate of patients' TMB in HCC.

Method: Following macrodissection and DNA purification, 48 samples derived from an international biorepository (21 fresh-frozen [FF] and 27 formalin-fixed paraffin-embedded, [FFPE]) underwent tNGS by OncomineTM Tumour Mutation Load Assay (1.5 Megabase exome coverage) on an Ion S5TM sequencer. We performed uracil-DNA glycosylase (UDG) pre-treatment in a group of 11 FFPE samples to verify its effect on fixation-induced cytosine deamination. In total, 30/48 samples satisfied post-sequencing quality control and were included for clinicopathological correlation. We classified samples as high/low TMB based on median number of mutations/Mb (Mut/Mb), testing different minimum allele frequency (MAF) thresholds (≥0.05, ≥0.1 and ≥0.2) in relationship with clinicopathological features including Overall (OS) and Recurrence-Free survival (RFS).

Results: Eligible patients (n=30) were mostly male, cirrhotic (84%) secondary to alcohol (48%) and Hepatitis C infection (45%). Median dominant tumour size was 4 cm with most patients being staged as TNM stage I-II (75%). Median OS was 12.3 months and median RFS was 12.1 months. FFPE samples displayed significantly higher TMB (median 958.39 vs 2.51 Mut/Mb, p<0.0001), estimated deamination counts (median 1335.50 vs 0, p<0.0001) and percentage C>T transition at CpG sites (median 60.3% vs 9.1%, p=0.002) compared to FF. UDG-treated FFPE samples carried significantly lower TMB (median 4019.92 vs 353 Mut/Mb, p=0.041), estimated deamination counts (median 6393.5 vs 328.5, p=0.041) compared to untreated FFPE. Adjustment of MAF to 0.1 and 0.2 reduced deamination counts (p<0.05), with a 0.2 threshold allowing for a reduction of TMB values within the interpretable range of <100 Mut/Mb in all samples. At a 0.2 MAF threshold with UDG treatment, the median number of non-synonymous mutations/Mb was 5.48 (range 1.68-16.07) and did not correlate with salient pathologic features of HCC including OS or RFS.

Conclusion: This pilot study highlights the challenges of TMB testing in archival tissue, where UDG pre-treatment and MAF adjustment to 0.2 allows for an improved assessment of TMB. Whilst tNGS on fresh HCC samples appears the optimal source of somatic DNA, the low median TMB values observed here may limit the role of TMB as a predictive correlate of response to immunotherapy in HCC.

P03-09YI Developing high drug-load arsenic trioxide microsphere for chemoembolization of hepatocellular carcinoma

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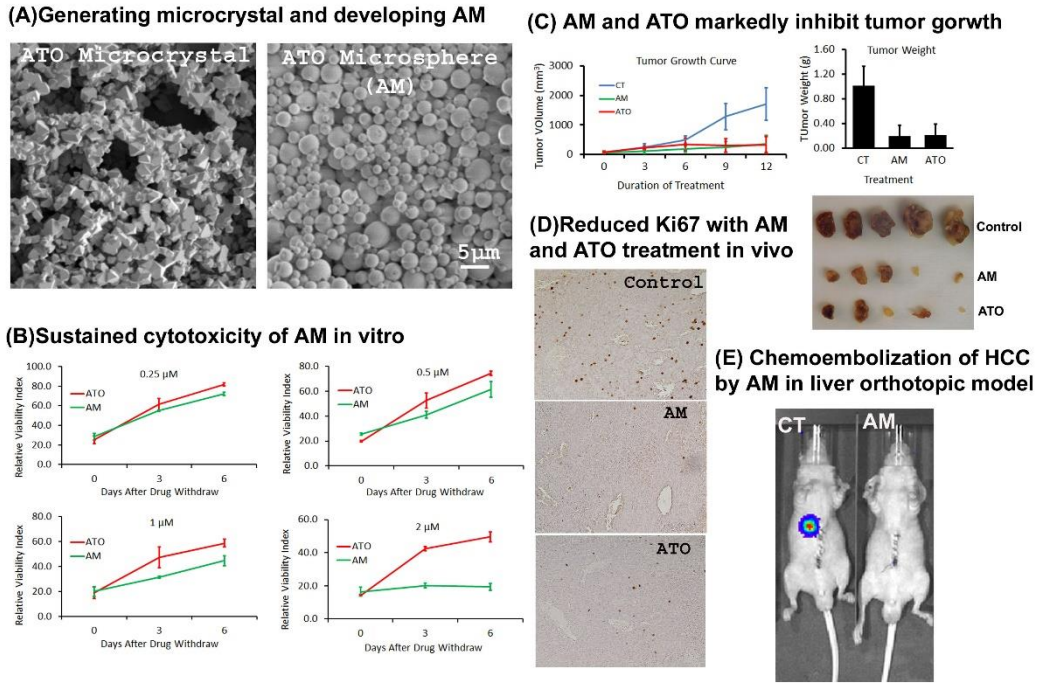
Background and aims: The effects of drug-eluting beads/microspheres in transarterial chemoembolization (TACE) for hepatocellular carcinoma (HCC) are quite variable due to multiple involved signaling and limited drug choices. Arsenic trioxide (ATO) is a chemotherapeutic agent involving multiple signaling and having substantial efficacy to HCC. But its usage is largely limited due to systemic toxicity. Using ATO-eluting beads in TACE can keep their local efficacy and avoid systemic exposure. However, sufficient drug-load for ATO beads, which is essential for local efficacy, is a challenging task so far. Through an innovative approach, we developed high drug-load ATO microspheres (AM) by using transient formed ATO microcystal as an intermediate.
Method: ATO microcrystal was formed by mixing ATO-NaOH solution with acetic acid under sonication. The microcrystal was encapsulated by poly (lactide-co-glycolic acid) using double emulsion to form AM. The effects of AM and ATO were tested by Calcein AM viability assay in vitro and intratumoral treatment on xenograft model. The chemoembolization function of AM was tested through portal vein injection (mimicking TACE) on liver orthotopic xenograft mouse model.
Results: Features of AM were: size 3.3 +/- 1.3 micrometer; drug load 40.1 +/- 8.5 %; and Zeta potential 40.41 +/-12.54 mV. Quick burst of ATO-eluting was at day 1 and sustained releasing plateaued at day 4. 36-hour treatment of 0.25 - 2 micromolar of AM and ATO showed cytotoxicity on Huh7 cells. ATO was slightly stronger. However, after drug withdraw, AM showed significantly higher cytotoxicity. By 6 days after drug withdraw, cells treated with 0.25, 0.5, 1 and 2 micromolar of AM were 12%, 17%, 24% and 61% less viable than ATO-treated. AM at 2 micromolar totally inhibited cell growth. In Huh7 subcutaneous xenograft model, intratumoral injection of AM and ATO at 100 microgram/kg inhibited tumor growth by 80% and 79% for tumor volume and 78% and 81% for tumor weight. Similarly, proliferation marker Ki67 was markedly reduced in AM and ATO treated tumors. In Huh7 liver orthotopic mouse model, portal vein administration of AM as a chemoembolization agent significantly inhibited tumor growth.
Conclusion: AM with drug load of 40.1% was generated, which was 4-10 folder higher than reported. AM had sustained drug effects and potent antitumoral activity to HCC. As a chemoembolization agent, AM markedly inhibited HCC growth in liver orthotopic xenograft model.

Figure:


(A)Generating microcrystal and developing AM
ATO Microcrystal
ATO Microsphere (AM)
5μm
(B)Sustained cytotoxicity of AM in vitro
0.25 μM
0.5 μM
1 μM
2 μM
ATO
AM
Relative Viability Index
Days After Drug Withdraw
(C) AM and ATO markedly inhibit tumor gorwth
Tumor Growth Curve
Tumor VOlume (mm³)
Duration of Treatment
Tumor Weight
Tumor Weight (g)
CT
AM
ATO
Treatment
(D)Reduced Ki67 with AM and ATO treatment in vivo
Control
AM
ATO
(E) Chemoembolization of HCC by AM in liver orthotopic model
CT
AM

P03-10 Evolution of indications for liver transplantation in the last 15 years: A single center experience in Madrid

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Background and aims: Liver transplantation (LT) is considered as the best treatment option for patients with end stage liver disease. Different factors, as changes in epidemiology or the introduction of new oral treatments for Hepatitis C (HCV), have an important influence in the type of transplanted patients

Method: Observational, retrospective analysis from prospective data collected from a single center in Madrid. We aimed to evaluate the changes in clinical characteristics of all transplanted patients from January 2005- march 2019. We divided into 3 periods of time: Period 1: years 2005-2009, period 2: 2010-2014 and period 3: 2015-2019

Results: We included 289 transplanted patients with a mean follow-up of 59 months (53-64)**.** LT due to HCV experienced an increased from 30.4% in period 1 to 43.8% in period 2 and a decreased to 25.9% in period 3 (p=0.004). Alcoholic liver-disease has been increasing progressively across the 3 periods (18% vs 29.2% vs 52.8%; p=0.004) as well as those-related to NASH (20% vs 20% vs 60%; p=0.004) and HIV co-infection (8.3% vs 33.3% vs 58.3%; p=0.004). On the other hand, HVB has decreased from 55.8% in period 1 to 30.8% in period 2 to 15.4% in period 3 and autoimmune-disease remained stable (38.1%) between period 1 and 3. Globally indication for hepatic decompensation has experienced an increase over the 3 periods (22.4% vs 33.6% vs 44%; p=<0.002), HCC has experienced a significant increase in period 3 compared to first and second (46.1% vs 29.2% vs 24.7%;p=0.002). In HCV population we found a significant decrease in patients transplanted due to decompensation in the 3 periods (40.6% vs 42.9% vs 34.5%), with a significance increase in HCC across the periods (40.6% vs 38.8% vs 65.5%) (p=0.09). In the first period, only patients with BCLC A were transplanted (n=29, 100%), in second and third period there was an increased in BCLC 0 (22.2% vs 42.9%) and decreased in BCLC B (75.6% vs 52.4%) (p=<0.001). Nodule size ≤2cm were more detected in the third than in second and first period (64.5% vs 33.3% vs 0%, p=<0.001). This finding is associated with an increase in the number of locoregional treatments in the third compared to first and second period (24.6% vs 36.8%, p=0.005)
Conclusion: Indications for LT are evolving following changes in epidemiology of liver disease. There is a significant direct and indirect impact of new treatments of HCV with an increase in HCC and a decrease in decompensated patients. HCC stage and nodule size has also decrease in the last 5 years

P03-11YI

Pilot study of transarterial radioembolization with Yttrium-90 in patients with hepatocellular carcinoma

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Background and aims: Transarterial radioembolization with Yttrium-90 (TARE) is applied at the initial, intermediate and advanced stages in patients with hepatocellular carcinoma (HCC). It has not demonstrated survival advantage over chemoembolization (TACE) or sorafenib and therefore it lacks a role in clinical guidelines. The aim of this study is to assess the efficacy and safety of TARE in HCC patients following a strict protocol at our center.

Method: Single-center pilot study of consecutive HCC patients treated with TARE between Dec-14 to Sep-19 following these indications: HCC not candidate to sorafenib; post-progression to sorafenib not candidate to regorafenib; downstaging for surgical resection. Clinical, analytical and imaging reviews were made at month 1, 3 and every 3 months thereafter.

Results: 30 patients were recruited: 15 sorafenib-naive, 10 post-sorafenib, 5 downstaging: 27 men, age 68.5 years, 27 with cirrhosis, alcohol (n = 17), HCV (n = 9) , Child A5 (n = 25); Child A6 (n = 2); healthy liver (n = 3); albumin 41.5 mg/dl, bilirubin 0.9 mg/dl, AFP 13.7 ng/mL; uninodular (n = 22), unilobar (n = 21), mean diameter 47.5 mm, BCLC-A (n = 1), BCLC-B (n = 9), BCLC-C [(n = 20), ECOG PS 1 (n = 2), segmental (n = 13) and lobar (n = 7) vascular invasion, no extrahepatic spread]. Median activity administered 2.45 GBq, median tumor dose 212 Gy. Median overall survival (OS) 17 months: 19 months (95% CI 3.432-34.568) in sorafenib-naive, 7 months (95% CI 0.000-14.176) post-sorafenib, not reached in downstaging (mean 20.5 months), p = 0.048. OS was different according to 1) distribution: unilobar 35 months (2.310-67.690) vs bilobar 11 months (0.000-22.687), p = 0.013; 2) number of nodules: single 35 months (6.278-63.722) vs multiple 13 months (0.000-31.259), p = 0.021; 3) radiological response at 3rd month (RECIST 1.1): absence of progression 35 months (9.116-60.884) vs progression 10 months (0.000-20.802), p = 0.023. There've been 5 complications: hematoma in femoral access (n=1), cholecystitis (n=2), pneumonia (n=1), radioembolization induced liver disease (REILD, n=1). 15 patients have died: listeria encephalomyelitis (n=1), REILD (n=1), tumor progression (n=13).

Conclusion: TARE is safe and effective in well-selected patients with HCC. Although applicability is low, those with uninodular disease, unilobar distribution and with absence of progression at 3rd month reach a median survival of 35 months.

P03-12YI Alpha Feta-Protein use as a standalone screening tool for hepatocellular carcinoma: Single centre experience

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Background and aims: The development of hepatocellular carcinoma (HCC) is a major complication of advanced liver disease. Current guidelines recommend screening with six monthly liver ultrasound and alpha-fetoprotein (AFP) in those at increased risk of HCC. Although AFP screening alone is not recommended due to poor sensitivity, it is performed in some centres due to the lack of availability of routine ultrasound. Weekly review of all AFP levels has been performed in our institution, in combination with ultrasound surveillance. AFP levels ≥10ng/ml are considered elevated, and trigger further case assessment. To determine the diagnostic yield of AFP screening for the diagnosis of HCC, and whether the identification of raised AFP levels impacted on clinical care.
Method: Retrospective analysis of all AFP levels reviewed in Beaumont Hepatology Unit over a 12 month period between 2017-2018. Patient data and demographics were collected from medical records
Results: A total of 1,365 AFP levels were reviewed in 2018, reflecting 994 patients. Of these 71 (5.2%) were elevated, relating to 39 patients. Of these 39 patients, disease aetiologies included hepatitis C (n=21/39), hepatitis B (n=8/39), hepatitis C/alcohol (n=3/39), alcohol (n=8/39), autoimmune hepatitis (n=1/39) and alpha-1 antitrypsin deficiency (n=1/39). 21 of 39 (54%) of patients had documented liver cirrhosis, while the stage of liver disease in 2/39 was unclear. Of the remaining 16 non-cirrhotic patients, 4 had HBV infection, and 1 was pregnant. The detection of an elevated AFP level triggered further investigation in 18 patients (46%); liver ultrasound was requested in 14, and axial (CT/MRI) liver imaging in 4. 14 patients had investigations booked as part of surveillance program prior to AFP results. Only one HCC case was detected in the 39 patients (2.6%) with elevated AFP levels, and a raised AFP assisted the diagnosis of HCC in only 0.1% patients in whom it was measured.
Conclusion: AFP levels should not be used as a standalone screening tool for HCC detection.

P03-13YI Feasibility of ultrasound-guided percutaneous biopsy in peri-hilar focal biliary lesions of the liver: A case series

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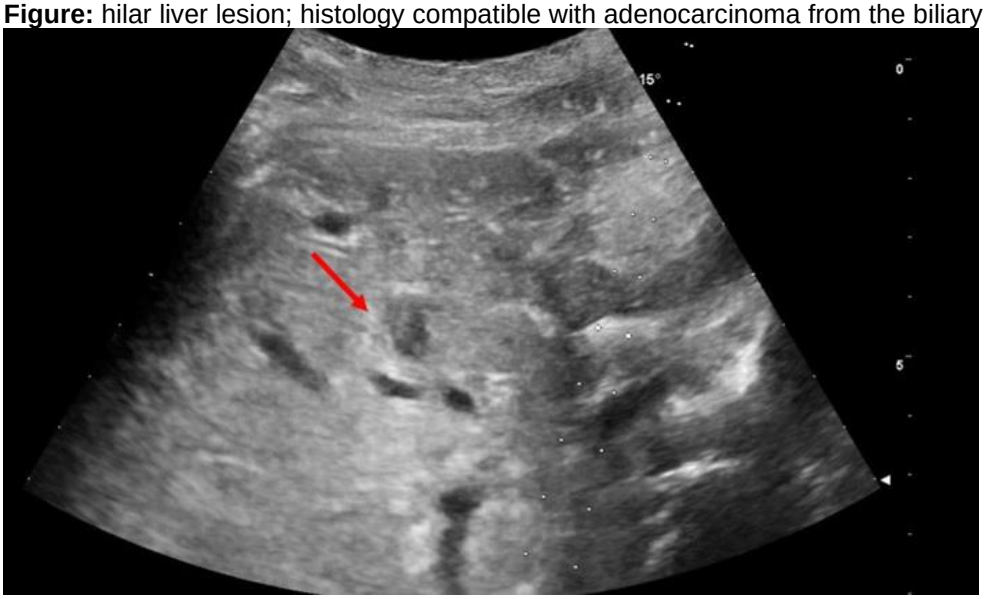
Background and aims: Radiological detection of a suspected peri-hilar lesion of the liver requires further investigation in order to exclude a mass-forming hilar cholangiocarcinoma. In patients not eligible for surgery, biopsy is mandatory for malignant confirmation. In these lesions, Ultrasound-Guided Percutaneous Biopsy (US-PB) has been, so far, poorly investigated. Our aim was to evaluate feasibility, diagnostic accuracy and safety of this technique.

Method: We included consecutive US-PB of peri-hilar, unresectable, focal lesions of the liver originating from the biliary tree. All procedures were performed with a 21G needle by an experienced hepatologist and radiologist from June 2018 to February 2019. For all the biopsies, the biliary convergence has been used as a landmark point; this area is visualized in front of the portal vein division. Data about anatomic site, diameter, radiological features from previous imaging, biomarkers, complications rate as well as tissue adequacy were retrospectively collected. We performed a descriptive statistical analysis.

Results: Ten patients underwent US-PB; 9 were included (male n=7, 77.8%), the exclusion was due to loss of follow-up. Mean size of the lesions was 30.3mm (range 15 – 52mm). The location was: (i) hilar in 4 patients, (ii) segment 3 (S3) in two, (iii) S2 in two and (iiii) S4 in one. Adequate tissue samples were obtained in all patients (mean sample diameter 1.07 cm, range 0.3 – 2.0cm): histological diagnosis of malignancy was observed in 6/9 patients (5 cholangiocarcinoma, 1 biliary metastasis from gastric carcinoma), while fibrosis was observed in three samples. In the latter group of patients, during a 6 month follow-up period, 2 patients showed no radiological changes (true-negative) while 1 patient showed disease progression (false-negative). The sensitivity of US-PB was 85.7% (95% CI 42.1-99.6%). In our cohort we did not observe any cases of early bleeding (within 4 hours from the US-PB), biliary fistula or seeding.

Conclusion: Ultrasound-Guided Percutaneous Biopsy for tissue sampling of peri-hilar liver lesion in patients not eligible for surgery is a safe alternative diagnostic technique.

Figure: hilar liver lesion; histology compatible with adenocarcinoma from the biliary tract



P03-14YI Dysregulation of aquaporin-1 in human CCA cells triggers epithelial-mesenchymal transition

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Background and aims: Cholangiocarcinoma (CCA) is a tumor arising from biliary epithelial cells. The overall prognosis is very poor due to the ability of the tumor to develop chemoresistance and metastasis. Epithelial-mesenchymal transition (EMT) is a multistep process during which epithelial cells gradually adopt structural and functional characteristics of mesenchymal cells. EMT is required for tumor cell migration, invasion and has been considered an early event of metastasis. EMT occurs in CCA where is associated with progression and chemoresistance. The function of aquaporin (AQP) water channels in tumors has been the focus of substantial research efforts. However, the role of AQPs in liver cancers and specifically in CCA remains unknown. Our aim was to study the role of aquaporin-1 (AQP1) in the regulation of EMT in CCA.

Method: Two established human CCA cell lines, HuCCT1 and KMCH, were used as experimental models. We generated KMCH AQP1 shRNA knock-down and HuCCT1 AQP1 KO by CRISPR/Cas9 system. Cell proliferation and migration were assessed by the Incucyte live-cell imaging system. We performed an unbiased discovering experiment using RNAseq comparing wild-type and AQP1 KO CCA cells. EMT and stemness markers expression were evaluated by RT-qPCR, western-blot and confocal microscopy

Results: Both CCA cell lines express AQP1 largely located in intracellular compartments. Dysregulation of AQP1 in tumor cells significantly induces proliferation and migration, suggesting a tumor suppressor role of AQP1 in CCA. RNAseq of AQP1 KO cells showed a total of 2702 differentially expressed genes. The Ingenuity Pathway Analysis showed Cancer (p-value range 3.36E-04 - 9.16E-34), Cellular movement (p-value range 2.54E-04 - 1.71E-20), and Cellular Growth and Proliferation (p-value range 2.23E-04 - 3.34E-11) as the top affected Diseases and Biological functions. In addition, the epithelial markers genes were significantly downregulated upon AQP1 silencing (E-cadherin gene *CDH1* by -133 fold change; $p < 0.001$, cytokeratin19 gene *KRT19* by -8 fold change; $p < 0.001$), while mesenchymal markers genes were significantly upregulated (vimentin gene *VIM* by 4 fold change; $p < 0.001$, fibronectin gene *FN1* by 3.51 fold change; $p < 0.001$), suggesting that the loss of AQP1 induced EMT. Furthermore, these changes were corroborated at mRNA and protein levels, with a major E-cadherin downregulation along with an upregulation of mesenchymal markers vimentin, fibronectin and EMT-inducing transcription factors ZEB1 and ZEB2.

Conclusion: Our data suggest that AQP1 acts as a tumour suppressor in CCA cells by acting both on proliferation and migration through an EMT process. Therefore, AQP1 might have an important role by restraining CCA progression.

P03-15YI Tracking neutrophils within the hepatocellular carcinoma microenvironment - development of relevant orthotopic and ex-vivo 3D liver-HCC culture models

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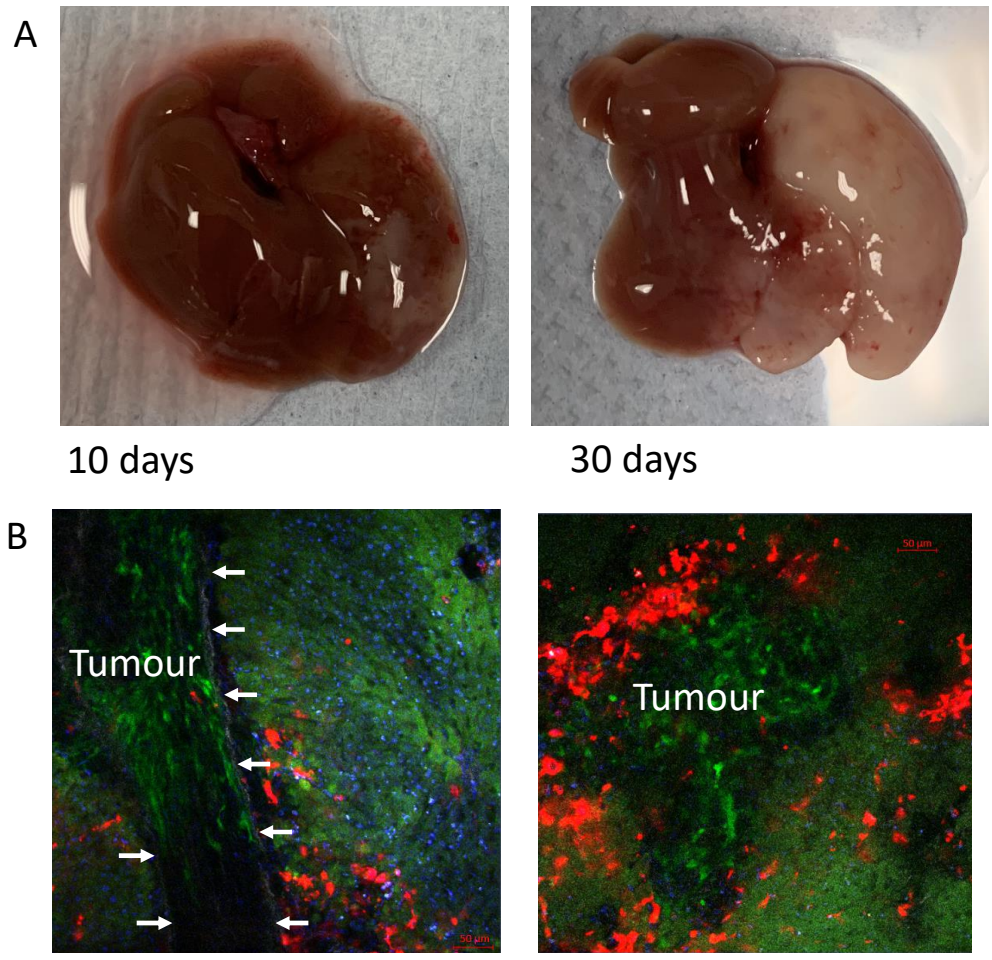
Background and aims: Immunotherapies have exciting potential in hepatocellular carcinoma (HCC), given that up to 50% of tumours have an immune component. Despite this, clinical studies indicate that only 15% of HCC patients respond to T cell immune checkpoint inhibitors. Targeting more than one component of the immune microenvironment may improve response rates and our previous work supports neutrophils as drivers of HCC and candidate immunotherapy targets. To better understand the complex role of neutrophils in HCC our aims were to develop models with an immune-rich tumour microenvironment, in which to explore the importance of neutrophils for tumour growth.

Method: Murine liver hepatoma Hep 53.4 cells were implanted via surgical intrahepatic injection into the left lateral lobe of wild type mice. A group of mice also underwent Ly6G antibody mediated neutrophil depletion prior to tumour implantation. Mice were harvested at 10 and 25 days and precision cut liver slice (PCLS) from cores of tumour, peri-tumour and non-tumour tissues created. Slices were kept in a bespoke PCLS bioreactor and rocked under incubation to ensure viability and retention of functionality. Isolated neutrophils were labelled for imaging and added to PCLS prior to multiphoton microscopy to visualise neutrophil uptake within the slices.

Results: Tumours were engrafted in all mice injected with Hep 53.4 cells and progressed up to 2cm in size at 4 weeks (figure A). On microscopic examination we noted substantive collagen deposition and stromal formation (figure B, white lines). FACS sorting of dissociated ex-vivo tumours revealed infiltration by multiple immune cell types including neutrophils. Neutrophil depletion significantly reduced tumour growth. PCLS from diseased livers survived for at least 5 days and added neutrophils migrated through the full thickness of the liver tissue, but preferentially accumulated in peri-tumour regions (figure B, neutrophils in red).

Conclusion: We have developed a new orthotopic model of HCC in mice that becomes populated by a variety of host immune cells. Tumours can also be incorporated ex-vivo in a PCLS-HCC model, designed for drug discovery and mechanistic investigations. The Hep 53.4 model has the advantage of its speed of set up and the opportunity to interrogate effects of manipulation of specific immune cell types including neutrophils, which were confirmed as important drivers of HCC.

Figure:



P03-16 Impact of treatment with tenofovir alafenamide (TAF) or tenofovir disoproxil fumarate (TDF) on hepatocellular carcinoma (HCC) incidence in patients with chronic hepatitis B (CHB)

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Background and aims: Potent antiviral treatment can reduce HCC incidence in CHB patients. TDF and TAF are first-line treatments, and in Phase 3 studies, TAF has shown antiviral efficacy similar to TDF, with higher rates of ALT normalization and no resistance. We evaluated the potential impact of treatment on HCC incidence in patients participating in the ongoing Phase 3 studies.

Method: HBeAg-positive (n=1053) and -negative (n=579) patients with HBV DNA ≥20,000 IU/mL and ALT >60 U/L (males) or >38 U/L (females) were recruited from 190 sites in 20 countries and randomized (2:1) to TAF 25 mg or TDF 300 mg QD for up to 3 years, followed by open-label TAF through Year 8. Patients with hepatic decompensation, co-infection with HCV/HDV/HIV, or evidence of HCC at screening were excluded. HCC was assessed by regular (every 6 month) hepatic ultrasonography introduced after Week 96 and throughout by local standards of care. Standardized incidence ratio (SIR) for HCC was calculated for observed cases relative to predicted risk using the REACH-B model.

Results: Through 5 years of follow-up, HCC occurred in 21/1632 patients (1.3%; TAF 11/1093 [1.0%]; TDF 10/539 [1.9%]). Median (Q1, Q3) time to HCC onset was 104 (55, 191) weeks (TAF 173 [56, 217], TDF 81 [26, 122] weeks). At baseline, relative to those without HCC, patients with HCC were more likely to be older (median age 53 vs 39 y; p<0.001), male (90% vs 65%; p=0.014), and more likely to be cirrhotic (FibroTest ≥0.75; 33% vs 9%; p<0.001). With treatment (TAF or TDF), HCC incidence was significantly reduced (SIR) [95% CI] 0.42 [0.27 -0.64]. For TAF-treated patients, a significant risk reduction was seen SIR [95% CI] 0.35 [0.19-0.62]. With TDF, there was a reduction in incidence, but it did not achieve statistical significance SIR [95% CI] 0.55 [0.30-1.02].

Conclusion: In CHB patients receiving TAF or TDF through 5 years, the incidence of HCC was reduced when compared to expected HCC incidence as determined by the REACH-B model. Additional follow up is needed to further characterize the impact of long-term treatment on HCC risk reduction.

P03-17YI E2F1 and E2F2 regulate glycerophospholipid metabolism in nonalcoholic fatty-liver disease-related hepatocarcinogenesis

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Background and aims: Nonalcoholic fatty-liver disease (NAFLD) has emerged as a risk factor for hepatocellular carcinoma (HCC). A shared feature of NAFLD and HCC is the altered glycerophospholipid metabolism. An altered hepatic phosphatidylcholine (PC) to phosphatidylethanolamine (PE) ratio is linked to NAFLD progression while increased biosynthesis of glycerophospholipids is coupled to activation of cell cycle. E2F1 and E2F2 have been both considered cell cycle activators and are upregulated in HCC; however, if they share a role controlling liver lipid metabolism in carcinogenesis is still unclear. Thus, the aim was to identify pathways by which both E2F1 and E2F2 modulate metabolism in NAFLD-related HCC.

Method: To induce HCC, *E2f1*$^{-/-}$, *E2f2*$^{-/-}$ and wild-type (WT) mice were treated with diethylnitrosamine (DEN) combined or not with a high-fat diet (HFD) until sacrificed at 9 months-old. Analysis of transcriptome, of liver lipid content and in vivo liver PC and PE synthesis were performed.
Results: The liver transcriptome analysis showed that the 19.9% of upregulated genes and the 33.3% of downregulated genes were related to metabolism in the NAFLD-HCC mouse model when compared to their control healthy mice. Among the downregulated pathways, those including glycerophospholipid metabolism were overrepresented. In concordance, the analysis of metabolic fluxes showed that PC and PE synthesis were decreased together with the PE concentration, making the PC to PE ratio increase. The lack of *E2f1* or *E2f2* protected mice from NAFLD-related HCC development. Genes involved in glycerophospholipid metabolism were upregulated in both *E2f1* and *E2f2*-KO mice livers. In fact, the upregulation of *Chpt1* and *Etkn2* expression was linked to increased synthesis of PC and PE. Besides, the increased liver PE concentration protected these mice from the observed increase in the PC to PE ratio in the NAFLD-related HCC. Interestingly, expression of *Pemt,* which transforms PE into PC, was also increased while PC content remained unaltered in both *E2f1* and *E2f2*-KO.

Conclusion: E2F1 and E2F2, both required for NAFLD-related HCC development, are involved in the rewiring of glycerophospholipid metabolism. The results suggest that the recovery of liver PE levels and reestablishment of the PC to PE ratio could benefit the therapeutical treatments.

P03-18YI Surveillance improves survival of intrahepatic cholangiocarcinoma arisen in liver cirrhosis

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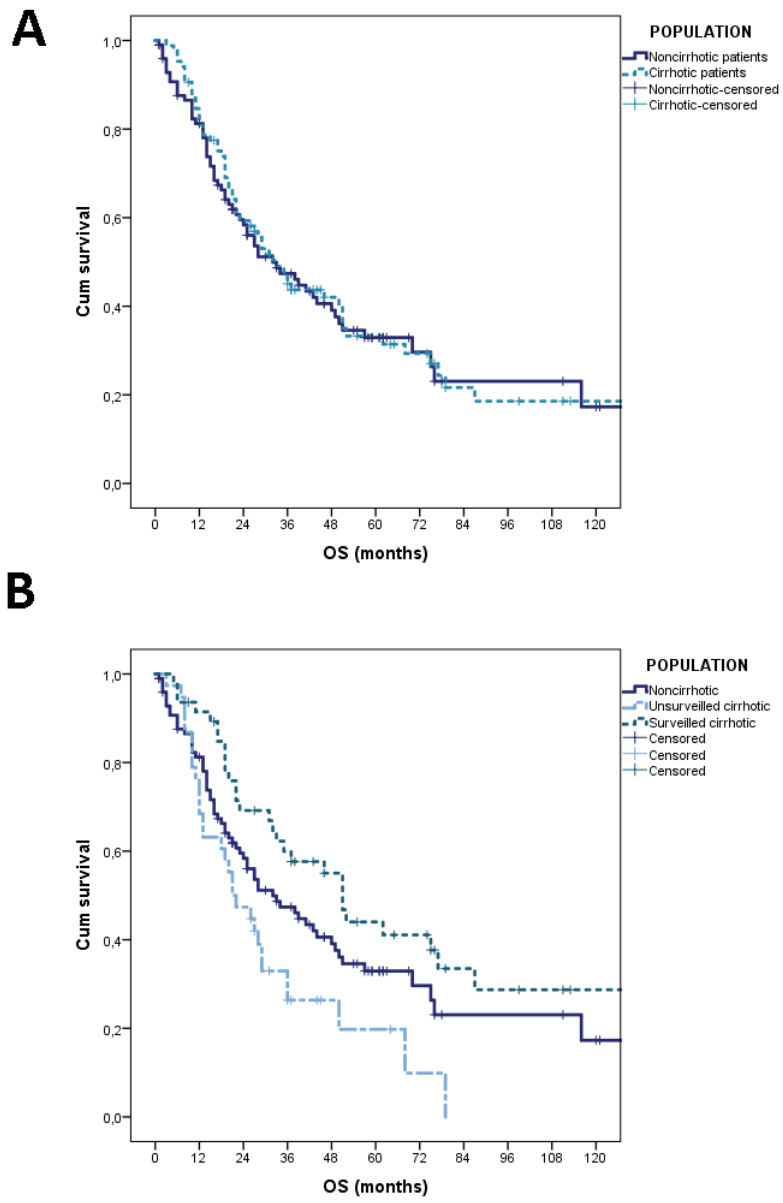
Background and aims: Due to its poor survival, intrahepatic cholangiocarcinoma (iCCA) is held to be a much more aggressive cancer than hepatocellular carcinoma (HCC), but in most series patients were diagnosed when symptomatic. However, iCCA is now increasingly being discovered during the surveillance for HCC in cirrhosis. Whether earlier detection of iCCA in cirrhosis is associated nonetheless with a dismal prognosis or not is unknown.

Method: Multicenter retrospective study of consecutively enrolled patients with histological diagnosis of iCCA. Patients were statified into subgroups according to the absence/presence of cirrhosis. A propensity score matching was performed to reduce the potential iases deriving form different baseline characteristic. Cirrhotic patients were further stratified according to their surveillance status. The lead-time bias was estimated and effects of the surveillance on the survival were adjusted accordingly.

Results: Data from 185 patients were gathered. Eighty-five patients (46.2%) were cirrhotic. Liver cirrhosis was not related to a worse overall survival (33.0 vs 32.0 months, p=0.800) even after the propensity score analysis (36.0 vs 43.0 months, p=0.227). Amongst the cirrhotic population,47 (55.3%) patients had received a diagnosis of iCCA during a surveillance program.The two subgroup differed in the tumour dimensions at the diagnosis (30 vs 48 mm in surveilled and nonsurveilled patients, respectively). As a result, surveilled patients were more likely to be treated surgically (59.8 vs 28.9%, p=0.003). OS was significantly different between surveilled and nonsurveilled patients (median overall survival 51.0 vs 21.0 months, p<0.001). The benefit of surveillance was confirmed after correction for the lead-time bias (adjusted hazard ratio 0.464 – 95% confidence interval 0.271-0.796).

Conclusion: Cirrhotic patients have a different clinical presentation and disease course of iCCA according to their surveillance status, significantly influencing the chance of receiving curative surgery and therefore of obtaining satisfactory long-term outcomes. The discrepant results of previous studies evaluating the prognostic role of cirrhosis might be at least partly justified by this aspect. In our series, cirrhosis (compensated in the majority of cases) was not associated with worse outcomes, and therefore cirrhosis itself should not discourage neither surgical nor and locoregional and systemic treatments.

Figure:


A
POPULATION
Noncirrhotic patients
Cirrhotic patients
Noncirrhotic-censored
Cirrhotic-censored
Cum survival
1,0
0,8
0,6
0,4
0,2
0,0
0
12
24
36
48
60
72
84
96
108
120
OS (months)
B
POPULATION
Noncirrhotic
Unsurveilled cirrhotic
Surveilled cirrhotic
Censored
Censored
Censored
Cum survival
1,0
0,8
0,6
0,4
0,2
0,0
0
12
24
36
48
60
72
84
96
108
120
OS (months)

P03-19YI Inactivation of the BAP1 tumor suppressor defines a subgroup of hepatocellular carcinoma with fibrolamellar features

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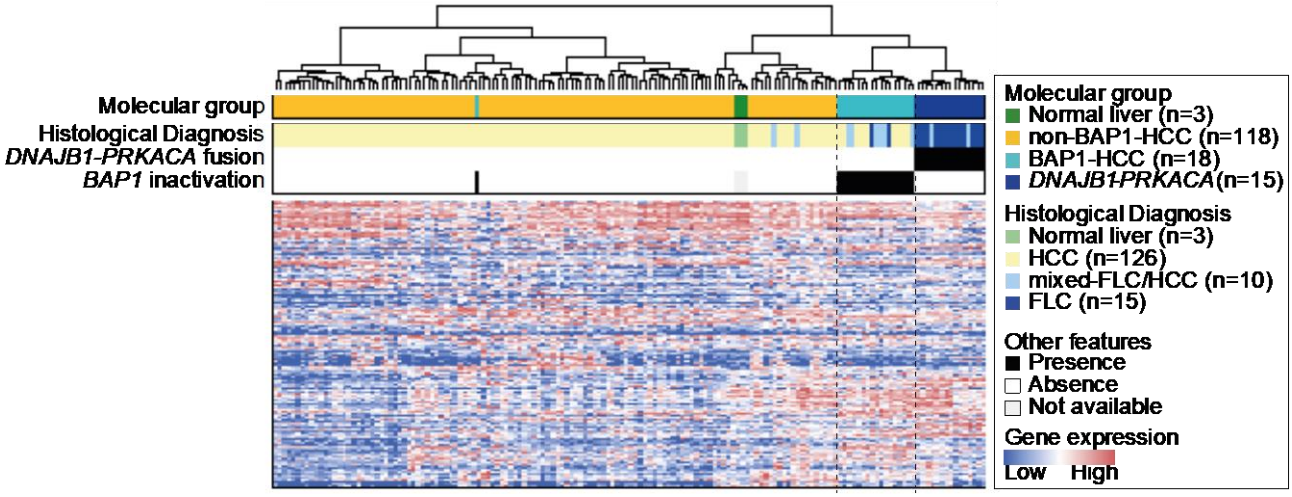
Background and aims: Fibrolamellar carcinoma (FLC) is a well characterized subtype of hepatocellular carcinoma (HCC) which was first defined by histological features and recently found to be driven by a recurrent *DNAJB1-PRKACA* fusion. However, liver tumors may harbour mixed histological features of HCC and FLC and these mixed-FLC/HCC still lack a comprehensive comparison with conventional HCC and FLC.

Method: We performed RNAseq and whole-genome- or whole-exome-sequencing in 151 liver tumors including 126 HCC, 15 FLC, and 10 mixed-FLC/HCC, with complete clinical and histological annotations. We used 340 HCC of the TCGA cohort for validation. Western-blot was performed to study the cAMP-dependent protein kinase (PKA) pathway.

Results: As expected, all FLC harboring the *DNAJB1-PRKACA* fusion clustered together (Figure), but we also observed a close cluster containing most of the mixed-FLC/HCC and characterized by a biallelic inactivation of the *BAP1* tumor suppressor (BAP1-HCC, Figure). BAP1-HCC patients were older and had a poorer prognosis compared to *DNAJB1-PRKACA* FLC patients, but otherwise they shared many features including enrichment in females, lack of chronic liver disease, high intratumor fibrosis or enrichment in progenitor markers. At the genomic level, BAP1-HCC showed a significant exclusion from mutations in classical HCC drivers such as *CTNNB1*, *TP53* and *TERT* promoter. BAP1-HCC lacked the *DNAJB1-PRKACA* fusion but nevertheless activated the PKA pathway through alternative copy-number alterations, showing recurrent gains / amplifications of the *PRKACA* locus together with a loss of the *PRKAR2A* locus (coding for the inhibitory subunit of PKA) thus leading to a high PRKACA/PRKAR2A ratio at the mRNA and protein levels.

Conclusion: *BAP1* mutated HCC encompass most of the mixed-FLC/HCC and constitute a specific subgroup of HCC sharing various clinical and histological features with *DNAJB1-PRKACA* driven FLC. This similarity may arise from a common over-activation of the PKA pathway in both groups of tumors.

Figure: Hierarchical clustering of liver tumors based on mRNA expression



P03-20YI Targeting antiapoptotic proteins Bcl-xL and Mcl-1 in cholangiocarcinoma

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Background and aims: Cholangiocarcinoma (CCA) is a highly chemoresistant malignancy arising from cholangiocytes with an increasing incidence and mortality. Among several mechanisms mediating chemoresistance, the overexpression of anti-apoptotic Bcl-2 proteins is of great importance. We investigated whether targeting antiapoptotic proteins with highly specific small molecule inhibitors could provide a new therapeutic strategy in CCA.

Method: Immunohistochemistry was used to analyze levels of Bcl-x_L and Mcl-1 in liver tissue samples from patients with intrahepatic cholangiocarcinoma (iCCA) or without this disease (control). Cell cycle and cell death were assessed by flow cytometry in CCA cells. In cell culture the small molecule inhibitors Wehi-539 (specific Bcl-x_L inhibitor), S63845 (Mcl-1 Inhibitor) and Abt-199 (Bcl-2 inhibitor) were used. Immunoblotting and q-RT PCR were used to investigate basaline expression of antiapoptotic proteins as well as their expression under treatment in CCA cell lines. RNA-sequencing and estimated protein activity employing the metaVIPER algorithm of a pan-cancer cohort were utilized to comparatively analyze antiapoptotic Bcl-2 protein activity.

Results: Tested CCA (n=32) samples had the highest Bcl-x_L transcript levels and estimated protein activity among all explored malignancies within the patient cohort (n>1500 cases). Mcl-1 and Bcl-2 showed comparably low activity in CCA. Bcl-x_L and Mcl-1 levels were elevated in iCCA tissue compared to cholangiocytes in matched and non-matched normal liver tissue. Expression of anti-apoptotic Bcl-2 proteins (Bcl-x_L, Mcl-1, Bcl-2) was also increased in the employed human CCA cell lines (HUCCT1, SNU1079, SNU478, SNU1196) compared to primary cultures of normal human cholangiocytes (NHC). Cell death was induced in all cell types after single treatment with Wehi-539 whereas the application of S63845 and Abt-199 did not result in that effect. Targeting both, Bcl-xL and Mcl-1, via a combination of Wehi-539 and S63845 lead to up to ten times higher cell death reaching >80% CCA cell killing.

Conclusion: Antiapoptotic proteins such as Bcl-x_L and Mcl-1 are upregulated and active in CCA. Combined treatment of Wehi-539 with S63645 showed a highly synergistic cell death inducing effect in CCA cells. This promising concept might be translated in clinical studies.

P03-21YI Protective role of aspirin chronic assumption in patients treated with sorafenib for hepatocellular carcinoma

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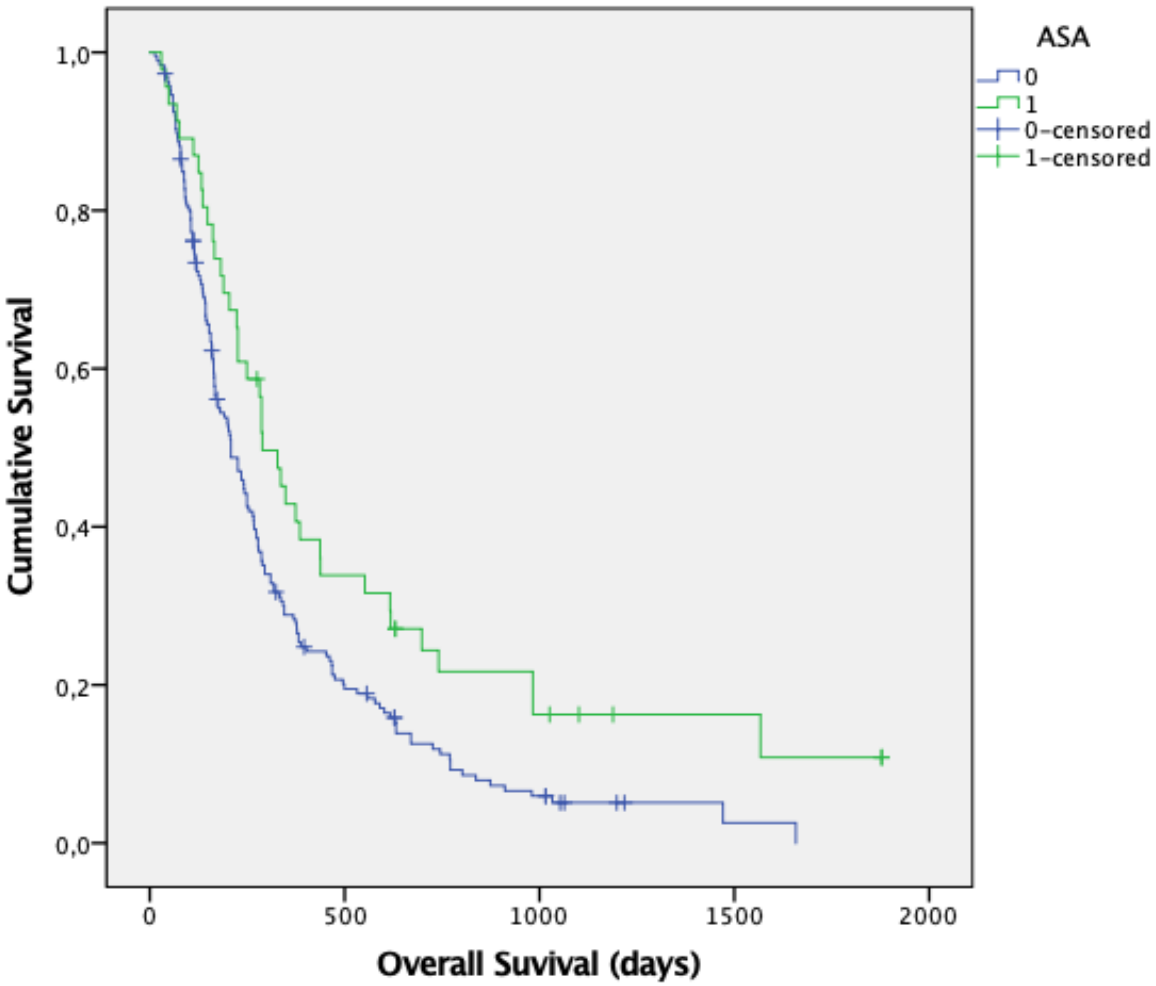
Background and aims: Hepatocellular carcinoma (HCC) is the most common primary liver cancer that usually develops in a microenvironment characterized by chronic liver inflammation. Several in vitro and mouse-model studies have shown the pivotal role of platelets in cancerogenesis, tumor cell migration and invasion. Platelets have also been reported to antagonize the effect of tyrosine kinase inhibitors such as sorafenib and regorafenib. The protective role of anti-platelet agents has largely been studied in different cancer types, including HCC. In particular, in vitro studies have shown the effect of aspirin in decreasing tumor cell proliferation, increasing sensitivity to chemotherapeutics and inducing apoptosis. All these findings suggest a possible synergy between the chronic inhibition of platelets activity by aspirin and sorafenib in patients with advanced HCC.
Method: We retrospectively analyzed baseline and follow-up clinical data of 232 patients who consecutively received sorafenib treatment from January 2008 and December 2017. Patients were divided in two groups according to the daily assumption of aspirin or not (46 and 186 patients, respectively). In order to evaluate the synergic activity of aspirin and sorafenib, in the OS analysis, patients who received a second-line treatment were censored when starting the new therapy.
Results: Baseline characteristics, adverse events and radiological response were consistent across groups. The OS survival of patients treated with aspirin was significantly higher (10.3 vs 7.4 months, p = 0.008). Multivariate time-dependent Cox regression analysis confirmed aspirin treatment as an independent protective factor (HR 0.658; 95%CI 0.460-0.941) when compared to other already established predictors of survival such as AFP > 400 ng/ml (HR 1.341; 95%CI 0.977-1.841; p = 0.070), Performance Status 1 (HR 1.320; 95%CI 0.951-1.830; p = 0.097), neoplastic thrombosis (HR 1.422; 95%CI 1.065-1.898; p = 0.017), extrahepatic spread (HR 1.227; 95%CI 0.915-1.646; p = 0.172) and dermatological adverse events (HR 0.579; 95%CI 0.437-0.767; p < 0.001).
Conclusion: Accordingly with data from pre-clinical studies, chronic assumption of aspirin seems to have a protective role also in patients with advanced HCC. A proven synergic effect between aspirin and sorafenib needs to be confirmed with further studies.

Figure:


ASA
0
1
0-censored
1-censored
Cumulative Survival
1,0
0,8
0,6
0,4
0,2
0,0
0
500
1000
1500
2000
Overall Suvival (days)

P03-22YI Impact of a Nurse Educational program for patients empowerment during sequential systemic therapy for hepatocellular carcinoma

Gemma Iserte[1], Neus Llarch[1 2], Víctor Sapena[1], Marco Sanduzzi Zamparelli[1], Sergio Muñoz Martinez[1], Jordi Rimola[3], Anna Darnell[3], Carmen Ayuso[3], Ernest Belmonte[3], Alejandro Forner[1], Jordi Bruix[1], María Reig[1]

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Email: giserte@clinic.cat

Background and aims: The BCLC Nurse Educational Program (BCLC-NEP; Llarch, ILC 2019) was designed for educational purposes. This program reduced on-site visits, optimized health resources and secured patient compliance, although 26% of the unscheduled phone calls visits (UPCVs) received during the early phase of treatment (ePT), which includes the first 60 days of sorafenib initiation, were related to administrative tasks. Other systemic treatments such as lenvatinib, regorafenib, cabozantinib and ramucirumab also require dose adjustments within the first 60 days of starting treatment, and the safety profile of each treatment is slightly different. The aim of this study is to evaluate the impact of the BCLC-NEP on regorafenib-treated patients and analyze whether the rate of administrative consultations differs in the second line setting.

Method: This is a retrospective study in regorafenib-treated patients in Hospital Clinic of Barcelona from 08/2016 to 12/2018. The BCLC-NEP includes an on-site educational appointment before starting regorafenib, on-site visits every month and UPCV. We collected the number, causes, type of issues raised by patients and the solution offered by the nurse team. UPCVs were divided according to their timing (early or late (lPT) after treatment start.

Results: The nurses received 170 UPCVs from all (n=21) but 1 patient who started regorafenib. We excluded 46 calls (27%) due to their administrative nature and 24 health-related UPCVs due to lack of information: We analyzed the remaining 100 UPCVs related to clinical issues; 42 calls from 19 patients were received in the ePT and 58 calls from 13 patients were received in the lPT. Analyzing the regorafenib related and non-related reported issues: the nurses solved 49 of the 100 UPCVs on their own.

Fifty-one of these 100 UPCVs were considered regorafenib related-adverse events (AE). All in all, the nurses themselves solved 14 (28%) of the regorafenib-related AEs during the ePT and 27 (54%) during the lPT.

Of all UPCVs received (n=170), 58 occurred during the ePT and 9 (15.5%) were related to administrative issues. Interestingly, this shows a 10.5% reduction as compared to the sorafenib population.

Conclusion: The BCLC Nurses Educational Program implemented for regorafenib-treated patient clearly shows the relevant role of nurses in this population (54% reforafenib-related AE calls were solved by nurses during the late phase). The dose-adjustments needed in the early phase and previous experience with sorafenib influence the nature of the calls received. The use of this educational tool during the systemic treatment sequence suggest a progressive empowerment of the patients and a more efficient use of resources.

P04-01YI Application of BCLC-B subclassification and the Hong Kong liver cancer systems to intermediate stage hepatocellular carcinoma

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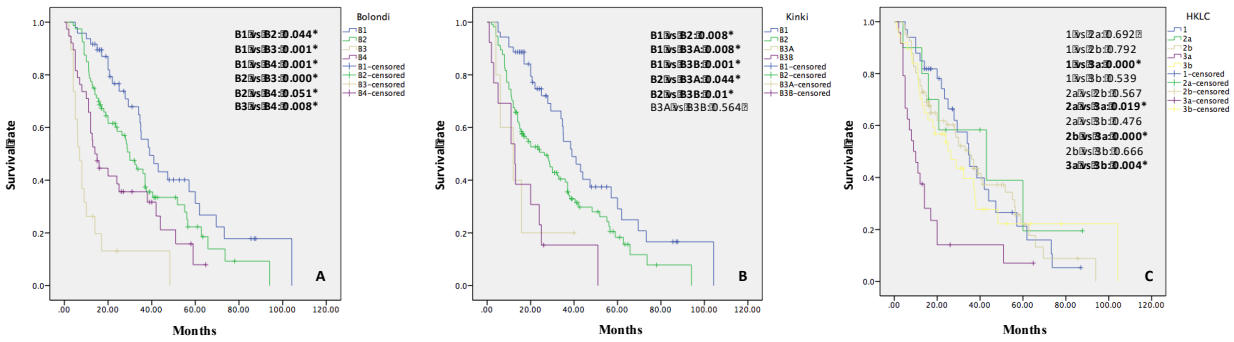
Background and aims: According to Barcelona Clinic Liver Cancer (BCLC), trans-arterial chemoembolization (TACE) is the recommended treatment for intermediate stage hepatocellular carcinoma (HCC) that is a heterogenous group in terms of tumor characteristics and liver disease stage. Since the single treatment option is limiting the adherence to CPG, several sub-classifications were proposed to increase the yield of intermediate stage HCC treatment. We applied two validated BCLC-B subclassifications and HKLC system to two Mediterranean HCC cohorts from Istanbul and Pisa to estimate the number of intermediate stage cases who could receive curative rather than palliative treatment options.

Method: We retrospectively reviewed the database of 1091 patients with HCC followed-up in a 10-year period (December 2008-June 2018). Patient, liver disease, tumor and treatment choice associated parameters were recorded. The Bolondi's and Kinki's sub-classification systems were calculated in each patient on the basis of the up-to-seven criteria, Child-Pugh score (CPS), and performance status. HKLC was classified according to the size and number of nodules, CPS, presence or absence of extrahepatic vascular invasion, and metastases. Survivals were calculated using Kaplan-Meier method, and the curves were compared using Log-rank test.

Results: One hundred and eighty-five (21.8%) patients had an intermediate stage HCC, 57 (30.7%), 58 (31.3%) and 124 (67%) of whom were candidates for curative treatments when using the Bolondi's (B1 and B4-up to 7), Kinki's (B1 and B3A) and HKLC (Stage I, IIA and IIB) systems respectively. Overall median survival was 28.7 months (95% Confidence Interval, 21.7-35.6). Both the Bolondi's and Kinki's sub-staging were able to discriminate survival probabilities for each category, besides the B3A vs B3B in the Kinki's system. Regarding the HKLC classification, a significantly lower median survival was observed for HKLC-IIIA in relation to the categories HKLC-I (p=0.000), HKLC-IIA (p=0.019), HKLC-IIB (p=0.000), HKLC-IIIB (P=0.004).

Conclusion: If HKLC was applied to intermediate stage HCC (BCLC-B) patients, approximately two-third of patients could be candidates for curative treatment. The Bolondi's BCLC sub-classification system showed the best performance in distinguishing survivals.

Figure: Survival comparison among BCLC-B sublassifications and HKLC stages



P04-02YI Curative versus palliative treatments for recurrent hepatocellular carcinoma: An italian nationwide study

Simone Famularo[1 2], Matteo Donadon[3], Federica Cipriani[4], Francesco Ardito[5], Marcello Maestri[6], Tommaso Dominioni[6], Davide Bernasconi[7], Francesca Carissimi[2], Maurizio Iaria[8], Maurizio Cosimelli[9], Giuliano La Barba[10], Sarah Molfino[11], Simone Conci[12], Cecilia Ferrari[13], Stefan Patauner[14], Antonio FLoridi[15], Marco Garatti[16], Adelmo Antonucci[17], Antonella Del Vecchio[18], Marco Chiarelli[19], Luca Fumagalli[19], Albert Troci[20], Andrea Percivale[13], Michela De Angelis[21], Enrico Lodo[22], Matteo Zanello[23], luigi Boccia[21], Michele Crespi[20], Riccardo Memeo[18], Giacomo Zanus[22], Giuseppe Zimmitti[16], Antonio Frena[14], Guido Griseri[13], Andrea Ruzzenente[12], Gianluca Baiocchi[11], Giorgio Ercolani[10], Gianluca Grazi[9], Raffaelle Dalla Valle[8], Elio Jovine[23], Felice Giuliante[5], Luca Aldrighetti[4], Guido Torzilli[3], Fabrizio Romano[2]

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Background and aims: Whether redo-surgery, thermoablation, trans-arterial-chemo-embolization or systemic therapy should be applied in patients with recurrent hepatocellular carcinoma (HCC) after primary surgery is currently unknown. The aim was to compare the Survival after Recurrence(SAR) of curative (RedoSurgery or ThermoAblation) versus palliative (Trans-arterial-chemo-embolization or Sistemic Therapies) treatments for recurrent HCC.

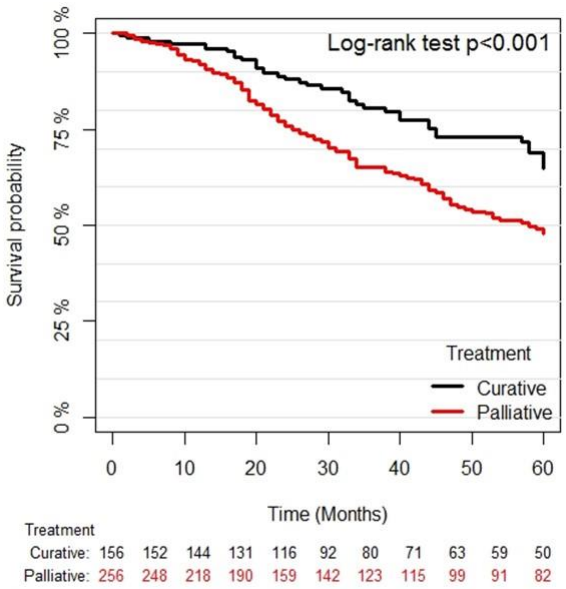
Method: Data were obtained from the surgical Italian register of HCC Recurrence (He.Rc.O.Le.S. Group), which collected aggregate data between 2008 and 2017 from 21 centers.Patients who experienced recurrence after surgery were included.Selected patients were then divided according to treatment allocation in Curative (CUR) or Palliative (PAL) Group. Inverse Probability Weighting (IPW) was performed to weight the groups and limiting the risk of bias.

Results: Among 1,560 patients in the register, 421(27%) experienced HCC recurrence and were included in this study:156(37%) in CUR and 256(63%) in PAL group. Tumor burden and liver function were weighted by IPW, and two pseudo-population were obtained (CUR=397.5 and PAL=415.38). SAR rates at 1-, 3- and 5-years were respectively 98.3%, 76.7%, 63.8 for CUR and 91.7%, 64.2% and 48.9% for PAL(p=0.007). Median DFS was 43 months(95%CI=32-74) for CUR group, while it was 23months(95%CI=18-27) for PAL(p:0.017). At the multivariate analysis, palliative therapies (HR=1.75;95%CI=1.14-2.67; p=0.01) and a recurrent HCC larger than 5 cm (HR=1.875; 95%CI=1.22-2.86; p=0.004) were the only predictors of mortality after recurrence. Time to recurrence per year of increase was the only protective factor (HR=0.616;95%CI=0.54-0.69; p<0.001).

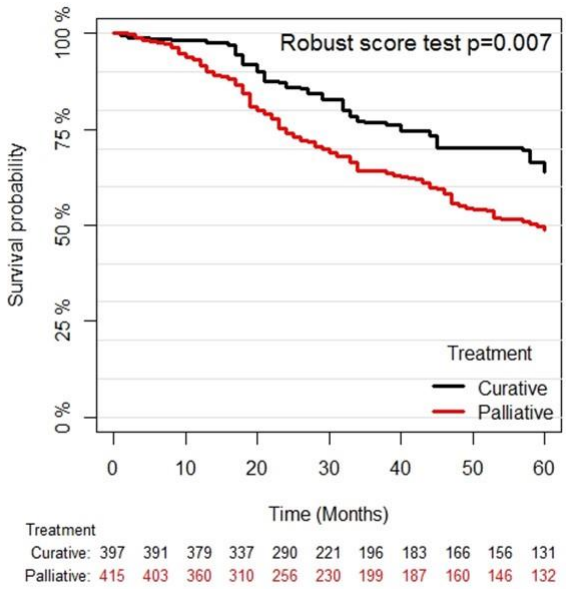
Conclusion: Curative therapies convey long-term survival in case of recurrent HCC developed after primary curative resection. Thus, surgery and thermoablation should not be avoided especially in case of limited tumor burden.

Figure:
Survival after Recurrence (SAR) curves before (A) and after (B) Inverse Probability Weighting.

A



B



P04-03 Association of single nucleotide polymorphisms for PNPLA3, Notch3 and EGF with hepatocellular carcinoma in alcohol-related liver disease

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Background and aims: PNPLA3 polymorphism is a known risk factor for hepatocellular carcinoma (HCC). Notch3 and EGF/EGFR signalling pathways play a role in carcinogenesis and their single nucleotide polymorphisms (SNPs) might contribute to HCC risk. The aim of this study was to examine the association of SNPs for PNPLA3, Notch3 and EGF with the HCC occurrence in alcoholic cirrhosis (AC).
Method: DNA was isolated from the whole blood of 189 AC transplant patients in the Merkur Liver Transplant Centre, Zagreb, Croatia. The groups consisted of 96 patients without HCC and 93, age and sex matched, patients with histologically proven HCC in the explanted livers. SNPs for PNPLA3 (rs738409), Notch3 (rs1043996) and EGF (rs4444903) were determined by PCR using commercially available TaqMan assays. Association between SNPs and HCC was examined in dominant, recessive, over-dominant and codominant models.
Results: Genotypes were in Hardy-Weinberg equilibrium (p = 0.66 for PNPLA3, p = 0.7 for Notch3 and p = 0.3 for EGF). The frequency of CC, CG and GG genotypes for PNPLA3 was 35%, 52% and 13%, respectively, in non-HCC group, and 24%, 44% and 32% in HCC group. GG genotype was associated with greater risk for HCC in recessive (OR 95%CI = 3.25 (1.54 - 6.86), p = 0.001), codominant (OR 95%CI = 3.75 (1.59 - 8.86), p = 0.004) and log-additive model (OR 95%CI = 1.86 (1.22 - 2.83), p = 0.003). For Notch3 the distribution of AA, AG and GG genotypes was 60%, 38% and 2%, respectively, in non-HCC group, and 51%, 40% and 9% in HCC group. No significant association with HCC was found, although, in log-additive (OR 95%CI = 1.57 (0.96 - 2.57), p = 0.066) and recessive model (OR 95%CI = 4.48 (0.92 - 21.67), p=0.054) the associations were close to the significance threshold. For EGF the distribution of AA, AG and GG genotypes was 35%, 49% and 16% respectively, in non-HCC group, and 27%, 57% and 16% in HCC group. Lack of association between EGF genotypes and HCC was found in any of the tested models (p>0.05). None of the SNPs was associated with the tumour size (PNPLA3 p = 0.19, Notch3 p = 0.63 and EGF p = 0.66) nor with the angioinvasion (PNPLA3 p = 0.65, Notch3 p = 0.39 and EGF p = 0.08).
Conclusion: PNPLA3 (rs738409) is associated with the risk of HCC development in patients with alcoholic cirrhosis, while EGF (rs4444903) is not associated. For Notch3 (rs1043996) the association was close to significant level and final conclusion may be given after inclusion of greater number of patients.

P04-04YI DKK1 drives cholangiocarcinoma growth through modulation of the immune microenvironment

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Background and aims: Dickkopf-1 (DKK1) is a secreted inhibitor of the canonical Wnt signalling pathway. It has been shown to be overexpressed in a number of cancers including cholangiocarcinoma (CCA), and is associated with worse outcomes in patients with the disease. This represents a paradox, where a Wnt inhibitor drives progression in what are primarily thought to be a Wnt driven cancer. Our research aims to understand this paradox by identifying novel pathways through which DKK1 promotes tumour growth in CCA.

Method: We use Hydrodynamic tail vein injection (HTVI) to induce specific genetic aberrations in wild-type mice leading to the development of CCA. With this model we are able to specifically overexpress DKK1 in tumours and model its effect on growth and on the tumour microenvironment. We use Nanostring gene expression analysis of tumour tissue to identify novel pathways modulated by DKK1 overexpression in this system. Using Co-immunoprecipitation mass spectrometry of HA-tagged DKK1 in CCA cell lines we are able to identify novel DKK1 binding partners in these cell lines use this to inform our understanding of DKK1's role in CCA.

Results: DKK1 overexpression drives increased tumour size in a HTVI model of CCA. In these tumours we are also able to show that DKK1 overexpression modulates the immune microenvironment, leading to an increase in chemokine expression and an increased number of tumour associated macrophages. DKK1 overexpressing tumours also show higher number of regulatory T-cells and gene expression changes indicative of an immune suppressive tumour microenvironment including reduced antigen presentation and co-stimulatory activation. Through Co-IP/MS we implicate CKAP4 as a potential DKK1 receptor and show that it is highly expressed in tumour associated fibroblasts in our *in vivo* model.

Conclusion: High levels of DKK1 in CCA are able to modulate the tumour microenvironment, facilitating tumour growth by driving a tolerogenic immune response. Identification of DKK1 binding partners suggests that binding to CKAP4 could represent a novel pathway through which DKK1 interacts with the tumour stroma. This is previously undescribed role for DKK1 in disease progression and may explain why the targeted inhibition of DKK1 shows efficacy in clinical trials. Future considerations should consider whether co-treatment with DKK1 targeted therapy and immunotherapies such as PDL1 inhibitors may represent an effective strategy in DKK1 overexpressing CCA.

P04-05YI Telomerase reverse transcriptase mutated circulating tumor DNA is a useful biomarker and predicts prognosis in Danish patients with hepatocellular carcinoma

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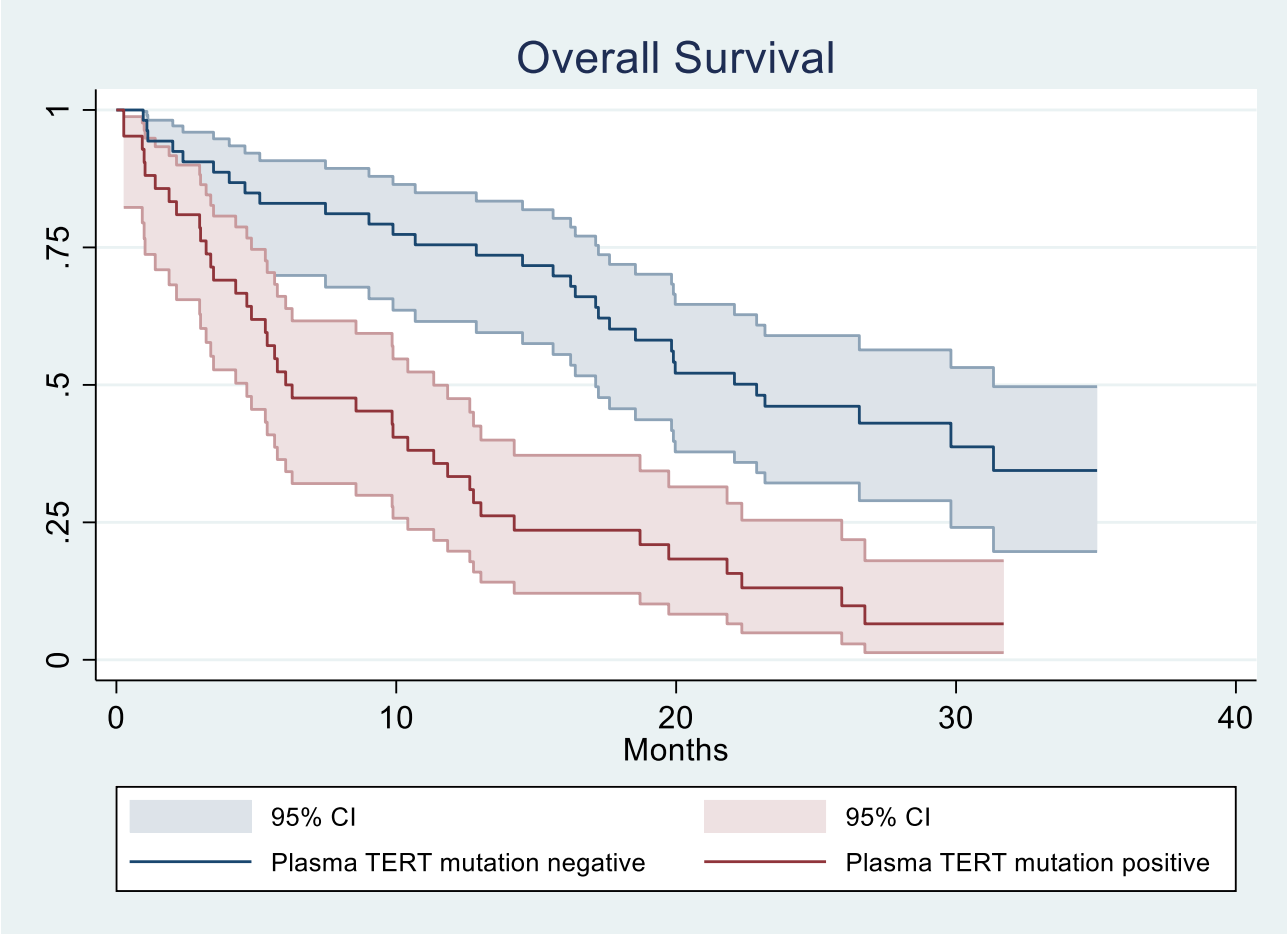
Background and aims: No biomarker is able to diagnose hepatocellular carcinoma (HCC) with high sensitivity and specificity or provide clinically useful prognostic guidance. Circulating tumor DNA (ctDNA) with tumor-specific mutations is an attractive biomarker. The *Telomerase Reverse Transcriptase* (TERT) C228T promotor mutation is the most prevalent tumor-associated mutation in HCC.
The mutational landscape of HCC depends on the etiology of any underlying liver disease. The aims of this study is to evaluate TERT mutation in plasma as a prognostic marker and evaluate presence of TERT mutation in a Danish patient cohort primarily consisting of patients with alcoholic cirrhosis.
Method: We analyzed plasma DNA from Danish patients, 95 with HCC and 44 with liver cirrhosis without HCC for the TERT promotor mutation using droplet digital PCR. In 34 HCC cases we also analyzed DNA from the corresponding primary tumors, and calculated the concordance rates. We investigated the association between presence of TERT mutation in plasma and tumor DNA and survival.
Results: Plasma TERT mutation was detected in 42/95 HCC patients (44%), but in none of the non-HCC patients. Moreover, TERT mutation was detected in 23/34 tumor samples (68%). In HCC patients, TERT mutation was associated with increased mortality when detected in plasma ctDNA (HR 2.27, P=0.005, adjusted for BCLC stage and sex), but not in tumor DNA (HR 1.39, P=0.52, adjusted for BCLC stage and sex). For HCC patients with BCLC stage C, we observed a difference in median survival of 10.4 months depending on plasma TERT mutational status (p=0.024).
Analysis of TERT mutations in plasma and tumor DNA from the same patient was concordant in 21/34 cases (62%; kappa value 0.31, P=0.014), 11 being double-positive and 10 double-negative. In the 13 non-concordant cases, TERT mutation was found in tumor DNA from 12 patients (92%) but not in the corresponding plasma DNA.
Conclusion: Plasma TERT mutation was found in 44% of Danish HCC patients and was associated with increased mortality. Moreover, plasma TERT mutation was specific for HCC patients, not being identified in non-HCC cirrhotic patients. We suggest TERT C228T mutation in ctDNA as a promising biomarker in HCC for both diagnosis and prognosis.

Figure:


Overall Survival
1
.75
.5
.25
0
0
10
20
30
40
Months
95% CI
95% CI
Plasma TERT mutation negative
Plasma TERT mutation positive

P04-06 Clinical outcome of lenvatinib therapy in Japanese patients with unresectable hepatocellular carcinoma ~a nationwide multicenter study~

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Background and aims: Lenvatinib (LEN) has been used in patients with unresectable hepatocellular carcinoma (u-HCC) who never experienced a systemic therapy or already received tyrosine kinase inhibitors (TKI) since Mar 2018 in Japan. We conducted a nationwide multicenter study.

Method: A total of 280 patients received LEN from Mar 2018 at 22 sites in Japan was enrolled. Tumour assessments in accordance with modified RECIST were done using dynamic CT or MRI within 4-8 weeks and every 6-8 weeks thereafter.

Results: Median age and body weight were 73 years and 60 kg. The baseline liver function was Child-Pugh class A in 223 (80%) patients and 154 (55%) patients were BCLC stage C. Median overall survival (OS) was not reached and 12-months survival rate was 57.0%. Median follow-up duration and overall time under treatment was 7.6 months and 3.7 months. Median progression free survival (PFS) was 8.5 months. Based on mRECIST, CR was shown in 13 (4%), PR in 69 (25%), SD in 83 (29%), and PD in 38 (14%) patients. Imaging data was not evaluated in 77 (28%) patients. Overall response rate (ORR) and disease control rate (DCR) were 29.3% and 58.9%. The patients who received lenvatinib as 1st line (TKI naïve, n=197) showed better survival than the patients who already received TKI (TKI experienced, n=83) (p=0.0002). The median OS in TKI experienced patients was 9.5 months and 12-months survival rate in TKI naïve patients was 65.5%. The TKI experienced patients significantly showed older age (p=0.008) and higher pretreatment ALBI score (p=0.002), however, there was no significant difference in pretreatment AFP level (p=0.21). 12-months survival rate in the TKI naïve patients with BCLC stage B and C were 66.3% and 62.8%. The frequent adverse events (AEs) during lenvatinb therapy were hypertension (44%), appetite loss (53%), and fatigue (48%), however, there was no significant difference in OS between the patients with and without these three AEs. The AEs which were associated with OS were liver associated AEs including increase of AST, ALT and T-Bil. The patients with liver associated AEs (n=67) showed poorer survival than the patients without such AEs (p<.0001). There was no significant difference in OS between the patients with and without hand-foot skin reaction (HFSR) during

lenvatinib therapy. The independent factors associated with OS in lenvatinib therapy were pretreatment performance status (HR 2.2, 95%CI 1.5-3.2, p<.0001), pretreatment ALBI score (HR 2.5, 95%CI 1.6-3.8, p<.0001) and TKI experience (HR 0.6, 95%CI 0.4-0.9, p=0.03).
Conclusion: Clinical outcome of lenvatinib therapy for Japanese patients with u-HCC in real world practice was similar to the phase 3 clinical trial, even though the Japanese patients were older and had lower bodyweight.

P04-07YI Umbelliferone loaded nano-lipidic carrier exerts diethylnitrosamine induced hepatic cancer via attenuation of cellular inflammation and cell proliferation

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Background and Aim: Among all cancer types, hepatocellular carcinoma (HCC) is the third most common cause of death. Clinical fraternity suggests that the only option for HCC is liver transplantation and surgical resection, both of which have limitations due to either late detection or patient condition.Targeted plant-based medicine is a good anti-cancer therapy option.In this research, we investigated the impact of umbelliferone loaded Nano-Lipidic Carriers (UF-NLCs) on diethylnitrosamine (DEN) induced hepatocellular carcinoma (HCC) by attenuating cellular inflammation and proliferating cells.
Methods: The intraperitoneal injection of DEN (200 mg / kg) was used to induce the HCC and rats received the oral administration of UF-NLCs. At the end of the experimental study, body weight, serum biomarkers, morphological and histopathological examination was performed. In order to investigate the possible mechanism, the inflammatory, pro-inflammatory, cancer preventive agent status, antioxidant enzymes and apoptosis marker were evaluated.
Result: UF-NLCs reduced hepatic nodules (83%), with a significant ($P<0.001$) increase in body weight (52.4%); altered hepatic marker levels viz., AFP (89.4%), AST (62.3%), ALT (61.7%), ALP (62.6%); non-hepatic parameters such as GGT (52.3%), albumin (55.3%), total protein (50.3%), BUN (58.9%), direct bilirubin (57.8%); Inflammatory cytokines such as TNF-α (63.4%),IL-6 (51.3%), IL-1β (56.6%) were significantly ($P<0.001$) down-regulated by UF-NLCs; inflammatory mediators such as COX-2 (49.4%), PGE2 (53.4%), iNOS (51.3%), and NF-kB (71.3%) compared to DEN control.
Conclusion: However, UF-NLCs demonstrated its chemoprotective role against DEN-induced HCC by attenuating cellular inflammation and cell proliferation.

P04-08 Stereotactic ablative radiotherapy in the management of metastatic and recurrent biliary tract cancer: Single institution analysis of outcome and toxicity

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Background and aims: Biliary tract cancers (BTC) are rare malignancies arising from biliary system. Systemic therapy is the cornerstone for stage IV disease, with about 12 months and 5 months of overall survival (OS) after first- and second-line treatment, respectively. Stereotactic radiotherapy (SRT) demonstrated to improve OS and progression-free survival (PFS) over standard of care in non-small-cell lung cancer, breast and prostate cancer. Evidence is lacking about safety and efficacy of local ablative treatments, such as surgery and SRT in the context of metastatic BTC (mBTC).
Method: We retrospectively analyzed the clinical outcomes for a cohort of mBTC patients treated at our institution with SRT for oligometastatic disease. Patients were included if in oligometastatic state as 1 to 5 distant metastases Analyzed outcomes included local progression free survival (LPFS), distant progression free survival (DPFS), PFS, and OS. Acute and late toxicities were scored with common terminology criteria for adverse events scale 5.0 (CTCAE 5.0).

Results: 51 patients meeting the inclusion criteria were identified from 2011 to 2018. Primary tumor sites were: 18 intrahepatic cholangiocarcinoma (35%), 16 extrahepatic cholangiocarcinoma (31%), 10 ampullary adenocarcinoma (20%), 7 gallbladder adenocarcinoma (14%). The majority of patients (26; 51%) received at least one line of systemic therapy prior to SRT. SRT targets were located as follows: 21 patients were treated on liver lesions, 17 on nodal metastasis, 5 patients on lung lesions, 4 patients on recurrence along the extrahepatic bile duct, 3 on synchronous liver and nodal metastases and 1 patient on bone metastasis. Twenty-six patients were treated for a single lesion (51%). After a median follow-up of 14 months median OS was 13.7 months, 1- and 2-year OS were 58% and 41%, respectively. Node and lung as metastatic sites were associated with a longer OS ($p<0.001$). Median PFS was 7.8 months, with 1- and 2-year PFS of 36% and 20%, respectively. Median LPFS was 26.8 months. Intrahepatic cholangiocarcinoma was associated with longer LPFS ($p=0.036$). Median DPFS was 11 months, with 1- and 2-year DPFS of 48% and 27.8%, respectively. At univariate analysis, patients treated for all metastatic sites had a trend to an improvement in DPFS ($p=0.051$). Ten patients reported grade 1-2 toxicity and 2 cases of acute G3 biliary obstruction requiring stent or percutaneous biliary drainage placement were registered in patients treated for node relapse along the biliary system.

Conclusion: SRT seems feasible in the context of mBTC. OS and PFS results are promising, considering that our patients were heavily pre-treated with systemic therapy before SBRT. Patients with nodal or lung relapse have a better prognosis. Distant relapses remain the main pattern of failure after SRT, but treatment of all metastatic sites seems to improve DMFS.

P04-09YI TREM2 protects the liver against hepatocellular carcinoma through multifactorial protective mechanisms

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Background and aims: Hepatocellular carcinoma (HCC) represents the 6th most common cancer world-wide and the 3rd cause of cancer-related mortality. HCCs arise in a background of chronic liver diseases where chronic inflammation and regeneration play a pivotal role. We have previously shown that the triggering receptor expressed on myeloid cells 2 (TREM2) protects the liver from hepatotoxic injury, via its negative regulation of toll-like receptor (TLR)-derived signalling in non-parenchymal liver cells. However, its role in liver cancer is still far from clear. Here, the role of TREM2 in hepatocarcinogenesis and liver regeneration was investigated.

Method: TREM2 expression was analysed in liver tissue of patients with HCC from 2 independent cohorts compared to control individuals. Wild type (WT) and *Trem2*$^{-/-}$ mice were subjected to experimental models of HCC and liver regeneration. *In vitro* studies with hepatic stellate cells (HSCs) and HCC spheroids were conducted.

Results: *TREM2* expression is increased in liver tissue of patients with HCC compared to normal liver tissue. Interestingly, TREM2 expression positively correlates with immune cell infiltration (macrophages, NKT cells, dendritic cells and B cells, among others) and activated HSC markers in human HCC tumours. In addition, *Trem2* expression was induced in the livers of mice subjected to DEN-induced carcinogenesis (a mouse model of HCC) and to partial hepatectomy (mouse model of liver regeneration). *Trem2*$^{-/-}$ mice developed more liver tumours after diethylnitrosamine (DEN) administration, which was associated with exacerbated liver damage, inflammation, oxidative stress and hepatocyte proliferation. Moreover, *Trem2*$^{-/-}$ livers showed increased hepatocyte proliferation and inflammation after partial hepatectomy. *Trem2*$^{-/-}$ mice also exhibited enhanced carcinogenesis in fibrosis-associated HCC models. Specifically, *Trem2*$^{-/-}$ mice displayed more small tumours after DEN + CCl4 injections and increased tumour volume after 40 weeks of TAA treatment. Administration of an anti-inflammatory diet blocked DEN-induced hepatocarcinogenesis in *Trem2*$^{-/-}$ mice. The supernatant of human hepatic stellate cells overexpressing TREM2 inhibits human HCC spheroid growth *in vitro*.

Conclusion: *TREM2* in non-parenchymal cells protects the liver from hepatocarcinogenesis, representing a novel promising therapeutic target.

P04-10YI Novel protein biomarkers in serum extracellular vesicles for the diagnosis of cholangiocarcinoma in patients with primary sclerosing cholangitis: A mirror of the tumour features

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Background and aims: Cholangiocarcinomas (CCAs) are heterogeneous malignant biliary tumors with very poor prognosis. Their etiologies are mostly unknown, but primary sclerosing cholangitis (PSC) constitutes a well-known risk factor. There are no accurate non-invasive methods for the early diagnosis of CCA. Extracellular vesicles (EVs) present in biofluids, have recently emerged as a potential source of biomarkers for several diseases. Here, we aimed to: 1) characterize the protein content of serum EVs from PSC-CCA patients and compare to patients with CCA of unknown etiology, PSC patients, and healthy controls, 2) determine the diagnostic accuracy of selected protein candidates, and 3) evaluate their expression in tumor tissue.

Method: Serum EVs were isolated from patients with PSC (n=39), CCA (n=30; unknown etiology), PSC-CCA (n=25) as well as from healthy individuals (n=41) by differential ultracentrifugation. The characterization of the EV fraction was performed by transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA) and immnunoblot. The protein content of EVs was determined by mass spectrometry-based proteomics and their diagnostic capacity (analysis of ROC curves with IBM SPSS statistics) was evaluated. Moreover, the expression (mRNA) of the selected protein candidates was evaluated in human CCA tumor and surrounding healthy tissue from two independent cohorts of patients (TCGA and Copenhagen), as well as in cultures of normal human (NHC) and tumor cholangiocytes.

Results: Isolated EVs presented a round morphology (TEM), similar diameter (~180nm; NTA) and typical markers of EVs such as CD9, CD63 and CD81 (immunoblot). By mass spectrometry, a total of 635 proteins were identified in serum EVs, and the proteomic analysis revealed a differential protein profile in patients with PSC, PSC-CCA, CCA, and healthy individuals. Certain identified proteins showed high diagnostic value for CCA (increased PIGR: AUC 0.96, decreased HEP2: AUC 0.87), and for the diagnosis of CCA in patients with PSC (increased FIBG: AUC 0.86 and decreased HEMO AUC 0.86). Of note, panels of selective biomarkers increased the diagnostic capacity. Importantly, some of these protein biomarkers present in serum EVs showed similar changes of expression (mRNA) in CCA tumor tissue and cells in culture compared to surrounding liver and normal human cholangiocytes, respectively, spotting their involvement in disease pathogenesis. Finally, several candidate protein biomarkers present in serum EVs are also detected in EV-derived from CCA cells compared to normal human cholangiocytes.

Conclusion: Serum EVs of patients with CCA contain specific proteomic signatures with high diagnostic capacity. Some of these biomarkers are specific for the diagnosis of CCA in patients with PSC, and others common for all CCAs. Certain biofluid biomarkers mimic their expression profile in tumor tissue.

P04-11 Does tumour size matter in intrahepatic cholangiocarcinoma? A population-based analysis

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Background and aims: The relationship between tumor size and survival in intrahepatic cholangiocarcinoma (ICC) was still controversial. This study aimed to explore the prognostic ability of tumor size for ICC.
Method: Between 2006 and 2016, 1004 ICC patients were selected from the Surveillance, Epidemiology, and End Results (SEER) Program. Using a cut-off point of 5 cm, patients were divided small tumor size group (STZ, diameter ≤ 5 cm) and large tumor size group (LTZ, diameter >5 cm). Kaplan-Meier analysis and Cox regression analysis were used to assess the prognostic ability of tumor size. Propensity score matching (PSM) was employed to balance the covariates. A patient cohort containing 325 ICC patients from the West China Hospital was used for validation.
Results: Among the studied patients, 471 were divided into STZ group and 533 into LTZ group. LTZ group was related to vascular invasion, poorer tumor differentiation, multiple tumors (all $p<0.05$). Patients in STZ group had better OS than those in LTZ group before and after PSM (all $p<0.001$). Subgroups analysis showed that 5 cm failed to classify ICC patients with T1 stage into T1a and T1b ($p=0.068$). Together with sex, N and M stage, tumor differentiation and vascular invasion, tumor size was proved to be associated with prognosis for ICC (Hazard ratio 1.402, $p=0.001$). Finally, diameter >5cm was also demonstrated to be a predictor of outcome for ICC in validation cohort (hazard ratio1.558, $p=0.021$).
Conclusion: Tumor size was an independent prognostic factor for ICC. Additionally, AJCC staging system using 5 cm to classify ICC patients into T1a and T1b may be not suitable.

Figure:

Figure_1
The workflow of selecting patients diagnosed with ICC.
ICC, intrahepatic cholangiocarcinoma; SEER, The Surveillance, Epidemiology, and End Results Program.

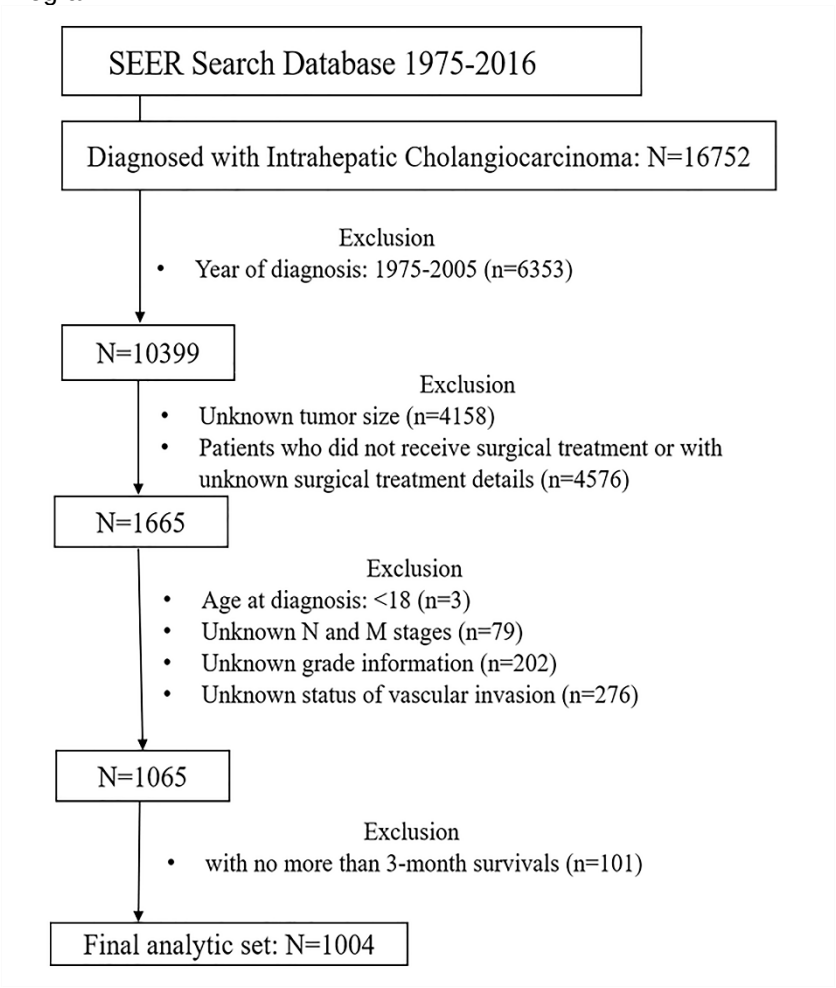


Figure_2
Kaplan-Meier analysis for ICC patients with a cut-off point of 5 cm in the SEER database
(A) before and (B) after PSM and in the West China Hospital patient cohort (C) before and (D) after PSM. OS, overall survival; ICC, intrahepatic cholangiocarcinoma; PSM, propensity score matching. Red lines represent patients with a tumor size ≤ 5 cm and green lines represent patients with a tumor size > 5 cm.

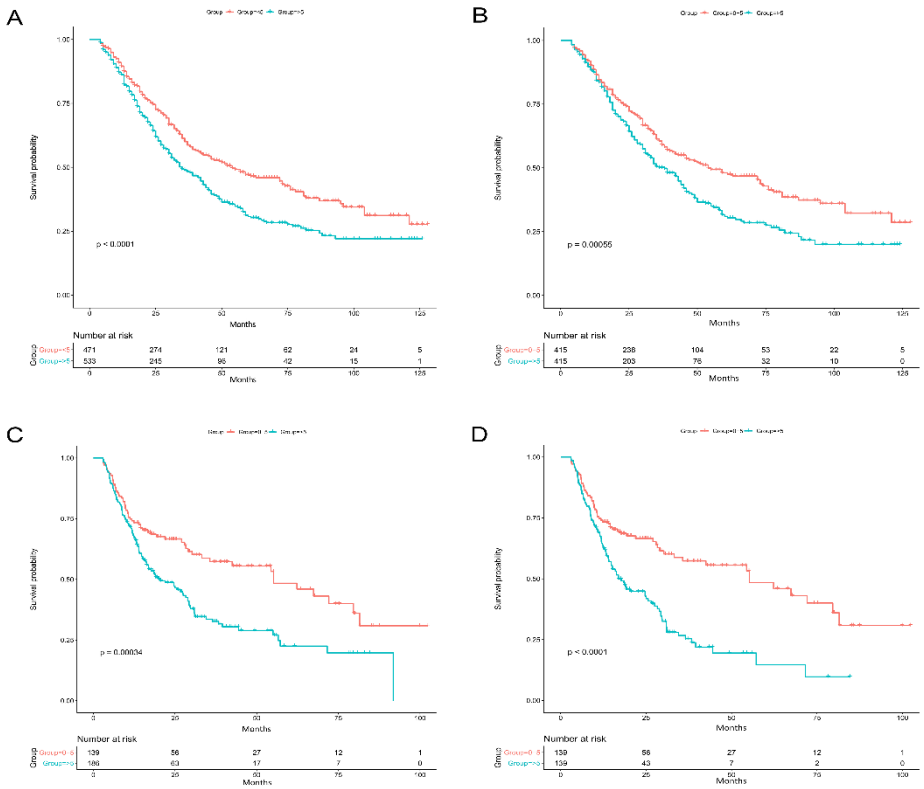


Figure_3
Univariate and multivariate Cox regression analysis of factors related to OS for ICC patients in the SEER database
(A) before PSM; (B) after PSM. OS, overall survival; ICC, intrahepatic cholangiocarcinoma; PSM, propensity score matching.

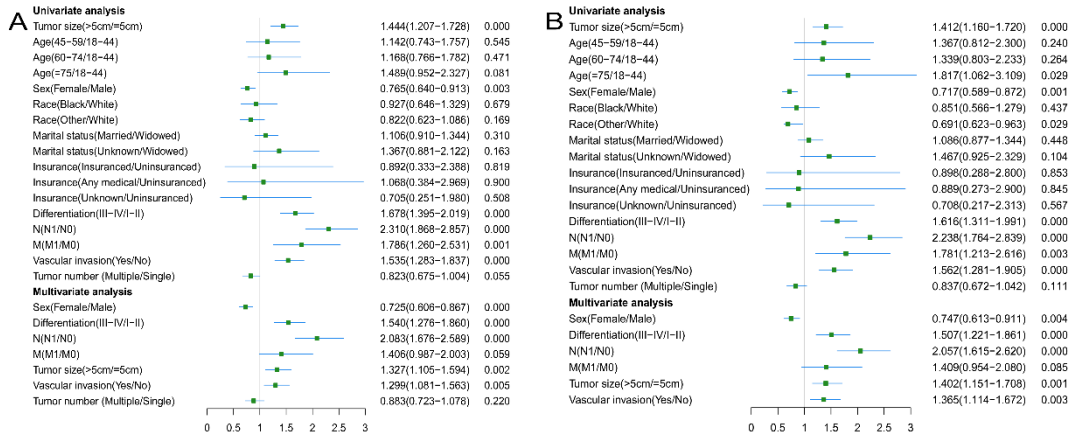
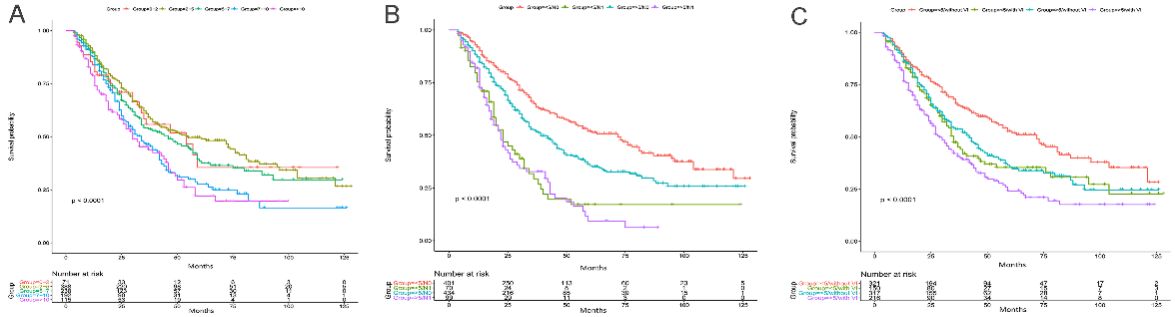


Figure_4
Subgroup analysis for different stratification criteria.
Kaplan-Meier analysis according to ICC size groups (A). Kaplan-Meier analysis for ICC size stratified by (B) N status and (C) vascular invasion. ICC, intrahepatic cholangiocarcinoma



P04-12YI c-Rel is a novel tumour suppressor and early prognostic indicator of hepatocellular carcinoma development

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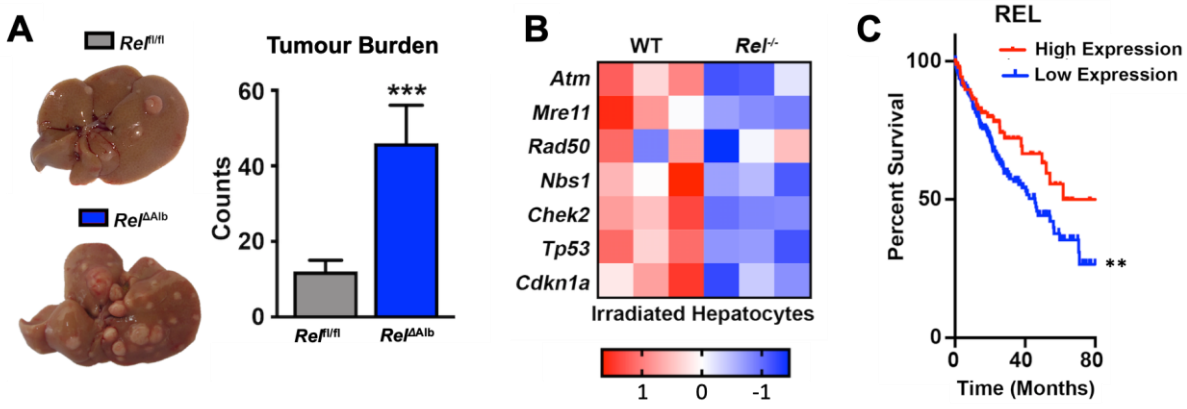
Background and aims: Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and is a leading cause of cancer-related death worldwide. The c-Rel subunit of the transcription factor NF-κB is widely considered to promote tumourigenesis. However, recently it has been described that c-Rel acts as a tumour suppressor in a murine model of B-cell lymphoma. We wish to discern whether c-Rel acts as a tumour suppressor in the development of liver cancer and understand the cellular mechanisms underpinning it.
Method: Wild type (WT) and global c-Rel knockout mice (*Rel*$^{-/-}$) were utilised as well as epithelial specific c-Rel knockout mice (*Rel*$^{\Delta Alb}$), generated by crossing Alb-cre mice with *Rel*$^{fl/fl}$ mice. The 30-week DEN model was used to induce hepatocellular carcinoma. An acute DEN injury model consisting of a single intraperitoneal injection of DEN was used to assess hepatic responses to genotoxic injury. Primary murine hepatocytes were isolated from global WT and R *Rel*$^{-/-}$. Publicly available HCC RNA-seq databases were interrogated for NF-κB expression, patient survival and mutational burden.

Results: Global *Rel*$^{-/-}$ mice develop more tumours than WT controls 30 weeks post DEN. The cell specific tumour suppressive role of c-Rel in the hepatocyte was confirmed in *Rel*$^{\Delta Alb}$ mice that also exhibited a significant increase in tumour number and stage compared to controls (Figure A). Underpinning this was an increase in cell death as a result of genotoxic injury with both *Rel*$^{-/-}$ mice and *Rel*$^{\Delta Alb}$ mice having increased liver damage, inflammation and compensatory proliferation following an acute dose of DEN.
Interestingly, primary murine hepatocytes isolated from *Rel*$^{-/-}$ were more susceptible to both DEN and ionising radiation-induced DNA damage. We show, for the first time, that c-Rel is a critical transcriptional regulator of the ATM-CHK2-p53 DNA damage response (Figure B) and propose that disruption of this pathway in *Rel*$^{-/-}$ mice drives genomic instability and tumourigenesis.
Examination of publicly available RNA-seq datasets highlights a previously unreported suppression of c-Rel in the tumours of patients with HCC. Kaplan-Meier survival curves that stratify patients based on NF-κB subunit expression, show that lower c-Rel expression is indicative of a more advanced disease and poorer prognosis (Figure C).
Conclusion: Our data provides the first evidence that c-Rel acts as a tumour suppressor in HCC and that c-Rel expression in early lesions may act as a biomarker to stratify patients with poorer prognosis.

Figure:



P04-13 Th therapeutic potential of adipose tissue-derived mesenchymal stem cells to enhance radiotherapy effects on hepatocellular carcinoma

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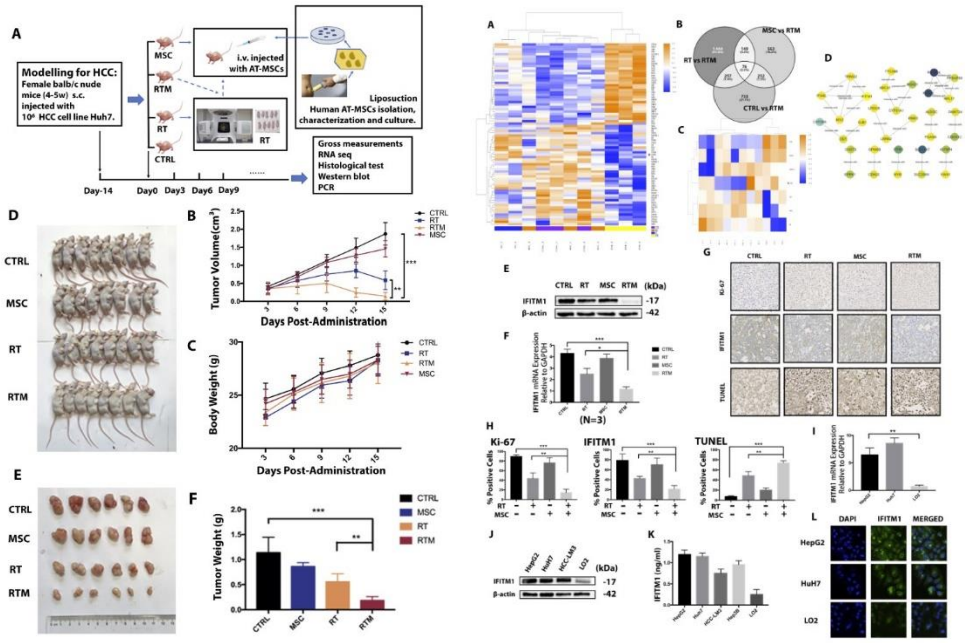
Background and aims: Because of the poor treatment efficacy, strong invasive activity and early metastasis, the survival rate of HCC patients remains low. Adipose tissue-derived mesenchymal stem cells (AT-MSCs) are one of the most promising types of mesenchymal stem cells, which can be easily obtained by minimally invasive procedures, and can differentiate into numerous cell lineages. Due to recent advancements in anticancer treatments, interests in the role of AT-MSCs in combating malignant diseases has grown. In the present study, we performed multiple experiments to evaluate the anti-cancer potential of AT-MSCs combined with RT on HCC cell lines-derived tumour models.
Method: Human AT-MSCs were isolated from the lipoaspirates of healthy donors undergoing elective liposuction. Two human HCC lines HepG2 and HuH7 were seeded. After incubation, the growth medium was replaced with non-conditioned control medium in the CTRL group, non-conditioned control medium followed by different doses of radiation in the RT group, AT-CM in the MSC group, or different doses of radiation followed by AT-CM in the RTM group. Cell proliferation, colony formation, sphere formation, wound healing, migration and invasion assays was analysed. For in vivo experiments, 5-week-old nude mice were injected subcutaneously with Huh7 cells. After the tumour volume reached ~100 mm3, the animals were randomized into four groups. RNA sequencing and high-throughput analysis were assessed to underlie the mechanisms in the synergistic effects of AT-MSCs and RT combination treatment
Results: Through direct co-culture and indirect separate culture experiments, we showed that AT-MSCs could enhance inhibitory effect of RT on reducing HCC cell growth, migration and invasion in both *in vitro* and *in vivo* experiments. RNA-sequencing analysis revealed a noticeable interferon-induced transmembrane 1 (IFITM1) -induced tumour gene signature. Gain and loss of mechanistic studies indicated that mechanism was attributed to downregulated expression of STAT3 and MMPs and upregulated expression of P53 and caspases. Collectively, our findings suggest that AT-MSCs might enhance the therapeutic effects of RT on HCC, providing a rationale for AT-MSCs and RT combination therapy as a new remedy for HCC.
Conclusion: Our data illustrated the therapeutic potential of human AT-MSCs on enhancing the treatment effects of RT on HCC.

Figure:


A
Modelling for HCC:
Female balb/c nude mice (4-5w) s.c. injected with 10⁶ HCC cell line Huh7.
MSC
RTM
RT
CTRL
i.v. injected with AT-MSCs
Liposuction
Human AT-MSCs isolation, characterization and culture.
Gross measurements
RNA seq
Histological test
Western blot
PCR
Day-14
Day0
Day3
Day6
Day9
B
Tumor Volume(cm³)
Days Post-Administration
C
Body Weight (g)
Days Post-Administration
D
E
F
Tumor Weight (g)
CTRL
MSC
RT
RTM
A
B
MSC vs RTM
RT vs RTM
CTRL vs RTM
C
D
E
IFITM1
β-actin
(kDa)
F
(N=3)
G
Ki-67
IFITM1
TUNEL
H
I
J
K
L
DAPI
IFITM1
MERGED
HepG2
HuH7
LO2

P04-14YI Serum lipidomic landscape of non-alcoholic fatty liver disease progression to hepatocellular carcinoma in a Caucasian population

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent (24% of population globally) and progressive liver disorder emerging as a leading risk factor for hepatocellular carcinoma (HCC). The molecular mechanisms behind the progression from NAFLD to HCC (NAFLD-HCC) remain elusive, and surveillance of 'at risk' NAFLD patients constitutes a clinical challenge. In this study, we elucidate the deregulated metabolic serum landscape of NAFLD-HCC patients and individual lipids diagnostic utility

Method: We have performed comprehensive ultra-high-performance liquid chromatography mass spectrometry (UHPLC-MS), investigating 1,295 metabolites in 196 serum samples from Caucasian patients with biopsy-proven diagnosis stratified into healthy and obese (CTRL=44), metabolic disease (NAFLD=93), NAFLD-HCC (n=27) and alcohol or viral-associated HCC (AV-HCC=32).

Results: In total, we detected 470 metabolites, including amino acids, their derivatives and lipids. We identified two major metabolic events in NAFLD-HCC. The initial metabolic rearrangement occurs in the onset of NAFLD (healthy liver; obese to NAFLD), characterized by differential expression (FDR $p<0.05$) of 232 metabolites (DEMs). The most prevalent metabolites impaired within the first metabolic step include amino acids, acylcarnitines (AC), bile acids, cholesteryl esters, and glycerophosphocholines. Contrary, the significantly augmented metabolic program covers mostly free fatty acids (FFA), as well as diglycerides and triglycerides (TG). The second metabolic rearrangement was observed at the neoplastic conversion from NAFLD to NAFLD-HCC. The late metabolic switch included major metabolic aberrations covering 334 DEMs with significant changes in AC, ceramides and FFA. The progressive change (Spearman, $p<0.05$) of metabolites showed gradual TG increase, progressive loss of AC and complete deterioration of FFA as the main perturbed metabolite subclasses. Multivariate analysis discriminated NAFLD-HCC patients from CTRL, NAFLD and HCC with a different etiology (AV-HCC) ($Q2=0.51$, $R2=0.57$). We generated ROC curves for DEMs and built a discriminator model including the top 5 metabolites, reaching a predictive accuracy >90%. The predictive power of the panel is superior to alpha-fetoprotein (AFP) and biochemical marker alanine transaminase (ALT) for the liver function:

	CTRL vs NAFLD-HCC	NAFLD vs NAFLD-HCC	AV-HCC vs NAFLD-HCC
Model	0.989	0.997	0.999
AFP	0.786	NA	0.613
ALT	0.776	0.733	0.570

Conclusion: Serum metabolomics revealed a specific perturbation of the lipid biology during the progression from healthy liver and morbidly obese, through NAFLD to malignant onset of NAFLD-HCC. Our metabolite panel can be clinically exploited in surveillance of patients at risk for developing HCC (morbidly obese, diabetic and NAFLD patients), and can distinguish NAFLD-HCC from AV-HCC.

P04-15YI Circulating levels of soluble urokinase plasminogen activator receptor (suPAR) predict outcome after resection of biliary tract cancer

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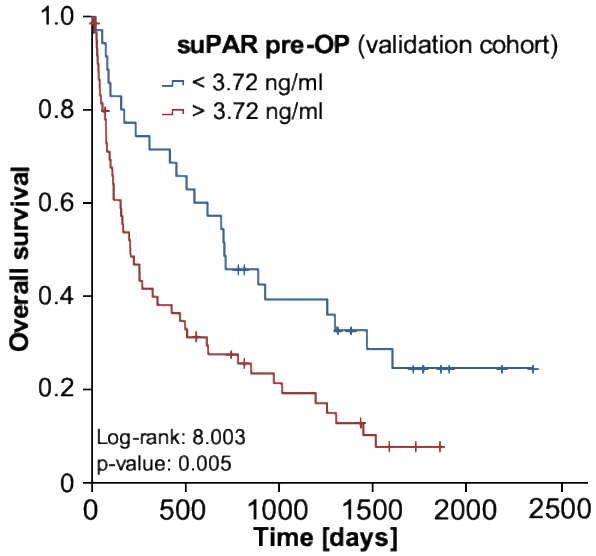
Background and aims: Surgical resection is the only curatively intended therapy for patients with biliary tract cancer (BTC), but 5-year survival rates after tumor resection have remained below 30%, corroborating the need for better preoperative stratification tools to identify the ideal surgical candidates. The soluble urokinase plasminogen activator receptor (suPAR) represents a mediator of inflammation and has recently been associated with cancer. In this study, we evaluated a potential role of suPAR as a novel biomarker in patients undergoing resection of BTC.

Method: Tumor expression of uPAR, the membrane bound source of suPAR, was analyzed by IHC in 108 BTC samples. Serum levels of suPAR were analyzed by ELISA in a training and validation cohort comprising a total of 117 BTC patients and 76 healthy controls.

Results: A high tumoral uPAR expression was associated with an adverse outcome after BTC resection. In line, circulating levels of suPAR were significantly elevated in BTC patients compared to healthy controls and patients with primary sclerosing cholangitis (PSC). Using a small training set, we established an optimal prognostic suPAR cut-off value of 3.72ng/ml for BTC patients. Importantly, preoperative suPAR serum levels above this cut-off value were associated with significantly impaired overall survival in both the training and validation cohort. Multivariate Cox-regression analysis including clinicopathological parameters such as the tumor stage, markers of systemic inflammation or organ dysfunction and established tumor markers revealed suPAR as an independent prognostic marker following BTC resection. Finally, high preoperative suPAR levels were indicative for acute kidney injury after tumor resection.

Conclusion: Circulating suPAR represents a previously unrecognized biomarker in patients with resectable BTC, which might be useful to preoperatively identify the ideal candidates for tumor resection.

Figure 1: Elevated levels of circulating suPAR are associated with an impaired overall survival after BTC resection. BTC patients (validation cohort) with preoperative suPAR level above 3.72 ng/mL have a significantly impaired overall survival.



P04-16YI Fatty acids regulate the biology of cholangiocarcinoma cells

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Background and aims: The incidence of cholangiocarcinoma (CCA) is increasing worldwide and is associated with poor patient outcomes. Identification molecular features of CCA could be helpful in designing new therapeutic approaches. Cancer cells are often exposed to a metabolically challenging environment with scarce availability of nutrients. This metabolic stress leads to changes in the balance between endogenous synthesis and exogenous uptake of fatty acids (FAs), which are needed by cells to support their own growth. Moreover, alterations in lipid metabolism may affect the response of tumor cells to different drugs. Yet, little is known about the lipid profile of CCA.
Method: CCA cells lines (CCLP1, HUCCT) were treated with increasing concentration of different fatty acids for 48h, and cell viability was evaluated. Proliferation and survival were evaluated with Western Blot analysis. Responsiveness of CCA cells to Oxalyplatin, Cisplatin and 5-FU was tested with crystal violet staining. The epithelial-mesenchymal transition program and stem-like markers were tested with real-time PCR.
Results: Exposure of both CCA lines to fatty acids led to a marked increase in cell proliferation, especially with oleic and palmitoleic acid. Western blot analysis demonstrated a robust activation of growth and survival pathways, including AKT and ERK1/2. In addition, exposure to fatty acids before treatment with and chemotherapeutic agents made CCA cells less sensitive to the toxic action of these drugs. Finally. Fatty acid treatment resulted in a marked upregulation of genes controlling epithelial-mesenchymal transition and key gene controlling stemness.
Conclusion: Our results indicate that CCA cells exploit lipid metabolism to gain growth, invasiveness and survival advantages. When exposed to fatty acids, cancer cells are more resistant to the toxic effects of antineoplastic drugs, show a modulation of stem-like features, indicating that lipid metabolism could be a new potential target to affect CCA progression.

P04-17YI Role of the tandem SOX17-MRP3 in the poor response of cholangiocarcinoma to chemotherapy

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Background and aims: A limitation for the treatment of cholangiocarcinoma (CCA) is its poor response to chemotherapy, which is partly due to the reduction of the intracellular levels of anticancer drugs through ABC pumps. Low expression of SOX17 has been associated with the malignant transformation of cholangiocytes. Whether SOX17 is also involved in CCA chemoresistance has been investigated.

Method: Viral vectors containing SOX17 ORF were generated to transduce CCA cells. Cell viability was determined by MTT test. Taqman Low Density Arrays (TLDAs) were used to measure mRNA abundance of ≈100 genes involved in chemoresistance. RT-QPCR, WB and IF were used to evaluate gene expression. Export activity of ABC pumps was determined by flow cytometry. Firefly luciferase (Luc2) was fused to ABCC3 promoter (ABCC3pr) to carry out promoter-reporter assays. The effect of SOX2 and SOX9 on ABCC3pr activity was analyzed by silencing with shRNAs. To analyze the ability of SOXs proteins to bind to ABCC3pr, EMSA was carried out. The impact of SOX17 expression on the efficacy of chemotherapy in vivo was evaluated with a subcutaneous xenograft model in mice.

Results: SOX17 expression in human CCA cells (EGI-1 and TFK-1) potentiated the cytotoxic activity of SN-38, 5-FU and mitoxantrone, but not that of gemcitabine, capecitabine, cisplatin or oxaliplatin. The analysis of the resistome by TLDAs revealed changes mainly affecting ABC pumps expression. Single-gene RT-qPCR, WB, and IF confirmed that MRP3 (highly expressed in human CCA tumors) was downregulated in SOX17-transduced CCA cells. The substrate specificity of this pump matched SOX17-induced selective chemosensitization in vitro. Functional studies showed lower ability of SOX17-expressing CCA cells to extrude specific MRP3 substrates. Promoter-reporter assay revealed that ABCC3pr activity was inhibited by SOX17 expression and silencing of SOX2 and SOX9. The latter was highly expressed in CCA. Moreover, SOX2 and SOX9, but not SOX17, induced altered electrophoretic mobility of ABCC3pr, which was prevented by SOX17. The growth of CCA tumors implanted into immunodeficient mice was inhibited by 5-FU. This effect was enhanced by co-treatment with adenoviral vectors encoding SOX17.

Conclusion: SOX9/2/17 are involved in MRP3-mediated CCA chemoresistance. Restored SOX17 expression, in addition to its tumor suppression effect, induces selective chemosensitization due to MRP3 downregulation and subsequent intracellular drug accumulation.

P04-18 Comparison between stratification system for liver cancer BCLC and HKLC-5 as a predictor of mortality in a university hospital cohort in Chile

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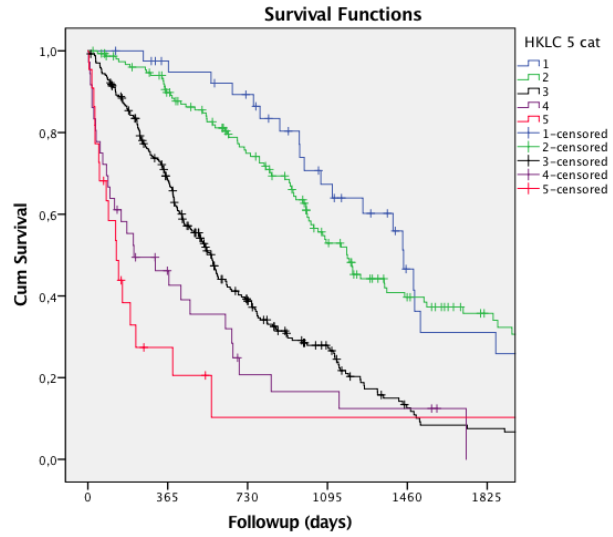
Background and aims: Hepatocellular carcinoma is growing arround the world. There are several stratification systems to predict mortality. We attempted to compare Barcelona Clinic Liver Cancer (BCLC) staging system and Hong Kong Liver Cancer (HKLC-5) staging system in a Latinoamerican population.

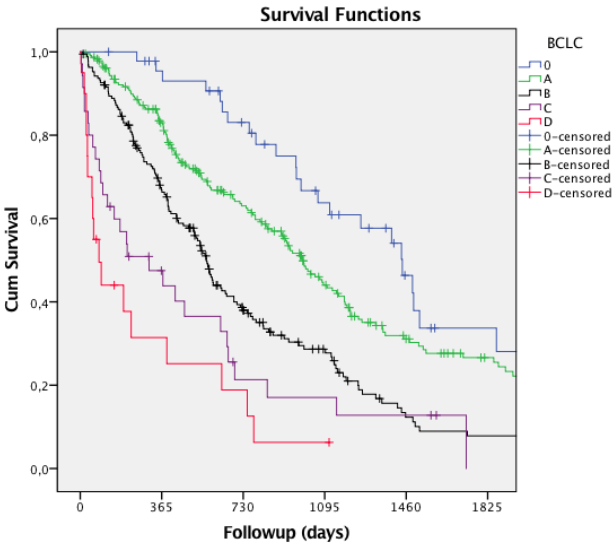
Method: We performed a retrospective analysis of data of patients who was diagnosis with HCC from January 2003 until July 2019. We exclude patients with resection or transplantation because they are considered potential curative therapies. We calculated HCC stage for each patient using 5-stage HKLC (HKLC-5) and the BCLC system. We compared performance of the BCLC and HKLC-5 systems in predicting patient outcomes using Kaplan-Meier estimates and AU ROC for ability to predict mortality at 6 month.

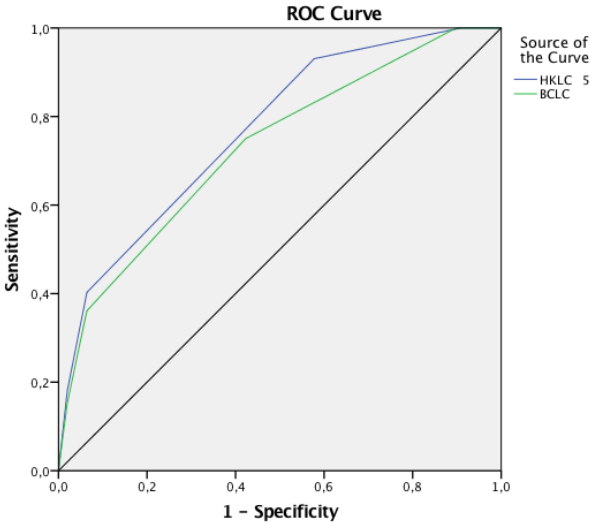
Results: We analysed 636 patients; exclude 75 patients with liver transplant and 31 who undergo surgery. Mean age was 65,9 ± 9,2 years; 63,9% were men. The principal aetiologies were NASH (45,6%), Alcoholic Liver Disease (19,7%), Hepatitis C (12,2%) and Cryptogenic (6,5%%). Median overall survival time, calculated from first image until date of death or censorship, for the entire cohort (all stages) was 659 days. The HKLC-5 and BCLC staging systems predicted patient survival times with significance (P < .001). HKLC-5 and BCLC each demonstrated good calibration with an AUROC of 0,77 (CI 95% 0,71 – 0,83) and 0,73 (CI 95% 0,71 - 0,83) respectively.

Conclusion: In a large cohort of Latin American patients who were diagnosed with CHC, we found that the HKLC-5 staging system has the best results in the separation of survival and discrimination, surpassing the BCLC system in predicting 6-month mortality.

Figure:


Survival Functions
HKLC 5 cat
1
2
3
4
5
1-censored
2-censored
3-censored
4-censored
5-censored
Cum Survival
1,0
0,8
0,6
0,4
0,2
0,0
0
365
730
1095
1460
1825
Followup (days)


Survival Functions
BCLC
0
A
B
C
D
0-censored
A-censored
B-censored
C-censored
D-censored
Cum Survival
1,0
0,8
0,6
0,4
0,2
0,0
0
365
730
1095
1460
1825
Followup (days)


ROC Curve
Source of the Curve
HKLC 5
BCLC
Sensitivity
1,0
0,8
0,6
0,4
0,2
0,0
0,0
0,2
0,4
0,6
0,8
1,0
1 - Specificity

P04-19YI Magnetic resonance of hepatocellular carcinoma treated with radiofrequency ablation: Radiomics to predict treatment response - preliminary results

Natally Horvat[1 2], Felipe Machado[3 4], Camila Tavares[1], Brunna Oliveira[2], João Vicente Horvat[1 2], Claudia Maccali[5 6], Anna Luisa Puga[4], Aline Lopes Chagas[5 6], Flair Jose Carrilho[5 6], Marcos Roberto Menezes[1 2], Antonildes N Assuncao Jr[3]

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Background and aims: Radiomics is an emerging field in medical imaging that extracts quantitative data from conventional radiological imaging modalities and correlate them with several clinical outcomes, including treatment response. The aim of this study is to investigate if quantitative textural features on contrast-enhanced MRI can predict local recurrence (LR) after radiofrequency ablation (RFA) in patients with hepatocellular carcinoma (HCC).

Method: In this IRB-approved study we retrospectively searched our maintained database for consecutive patients who underwent RFA due to probable or definitive imaging diagnosis of HCC (LI-RADS 4 or 5), from July 2011 to May 2018. The exclusion criteria were: (a) patients without pretreatment MRI; (b) an interval between pretreatment MRI and RFA > 60 days; (c) patients without follow-up MRI or surgical specimen within at least 1 year after RFA, (d) previously treated nodules, and (e) incomplete response after RFA. One experienced radiologist manually segmented the hepatic nodules in all slices on arterial and delayed phases using ITK-SNAP v3.4.0. One hundred and seven radiomic features were extracted using Python v3.6 consisting of: first-order features (n=18), 3D shape-based (n=14) and second-order features (gray level co-occurrence matrix (GLCM, n=24), gray level run length matrix (GLRLM, n=16), gray level size zone matrix (GLSZM, n=16), neighboring gray tone difference matrix (NGTDM, n=5) and gray level dependence matrix (GLDM, n=14)). Statistical analysis was performed to evaluate associations between radiomic features and LR, defined as an abnormal area of enhancement within the treated area on MRI or viable tumor on surgical specimen.

Results: This study consisted of 34 patients (mean age 67 ± 9 years, 70% male) with 51 HCC treated hepatic nodules. Thirteen nodules (25%) had LR. There was no significant difference on clinical and laboratorial findings of nodules with and without LR. Among radiomic features, 32/107 features (30%) extracted from arterial phase had AUC > 0.7. The vast majority of them, 24/32 (75%), were second order radiomic features. None of the features extracted from delayed phase obtained AUC > 0.7. The radiomic model using 5 principal components obtained an AUC of 0.78, sensitivity of 0.73, specificity of 0.81, PPV of 0.70, and NPV of 0.73 in predicting LR.

Conclusion: Our results demonstrate that radiomics on pre-treatment MRI can predict patients who will exhibit LR of HCC after RFA.

P04-20YI PARP-1 inhibition preferentially impairs KRAS-mutated intrahepatic cholangiocarcinoma and induces distinct molecular alterations

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Background and aims: Activating KRAS mutations are among the most abundant genetic alterations in intrahepatic cholangiocarcinoma (iCCA) and are associated with early recurrence, poor therapeutic response and reduced overall survival. Poly (ADP-ribose) polymerase 1 (PARP-1) expression is frequently observed to be upregulated in iCCA and associated with DNA damage response, cell death signaling, inflammation and changes in cell metabolism. Experimental evidence indicates a potential therapeutic relevance for PARP-1 inhibition in iCCA that preferentially affects KRAS-mutated cancers, but the exact molecular mechanisms of the interaction remain unknown.

Method: CRISPR/Cas9-mediated knockout and treatment with PARP-1-inhibitor AZD2281 were conducted in several KRAS-mutated and non-mutated iCCA cell lines. Assessment of PARP-1 knockout and inhibition on tumorigenic potential was analyzed by viability assay and colony and sphere formation. RNA Sequencing of CRISPR/Cas9 PARP-1 knockout clones was employed to further analyze underlying molecular pathways.

Results: A significant upregulation of PARP-1, as well as enrichment of a gene set related to PARP-1 activation, was observed in iCCA tissue and RNASeq data compared to control, indicating a potential role of a PARP-1 signature in cholangiocarcinogenesis. Interestingly, the investigation of PARP-1 expression showed higher levels in KRAS-mutated compared to non-mutated cell lines. Consistently, knockout of PARP-1 preferentially impaired in KRAS-mutated cell lines and led to a 40-45% reduction in colony and sphere formation, besides sensitizing KRAS-mutated cells towards DNA double-strand break-inducing agents. Also, KRAS-mutated cell lines were significantly more sensitive to treatment with PARP-1 inhibitor. RNA Sequencing analysis of CRISPR/Cas9 PARP-1 knockout clones employing GSEA and IPA revealed differential expression of DNA damage response pathways as well as other cellular pathways known to be affected by PARP-1, e.g. inflammation, c-MYC and cell death signaling.

Conclusion: These investigations confirm a preferential sensitivity of KRAS-mutated iCCA towards PARP-1 based interventions suggesting an unrecognized therapeutic role in this poor prognostic subgroup of iCCA patients.

P04-21Y Inhibitory effects of butyrate carrier structured lipids on persistent preneoplastic lesions induced by the resistant hepatocyte model of hepatocarcinogenesis

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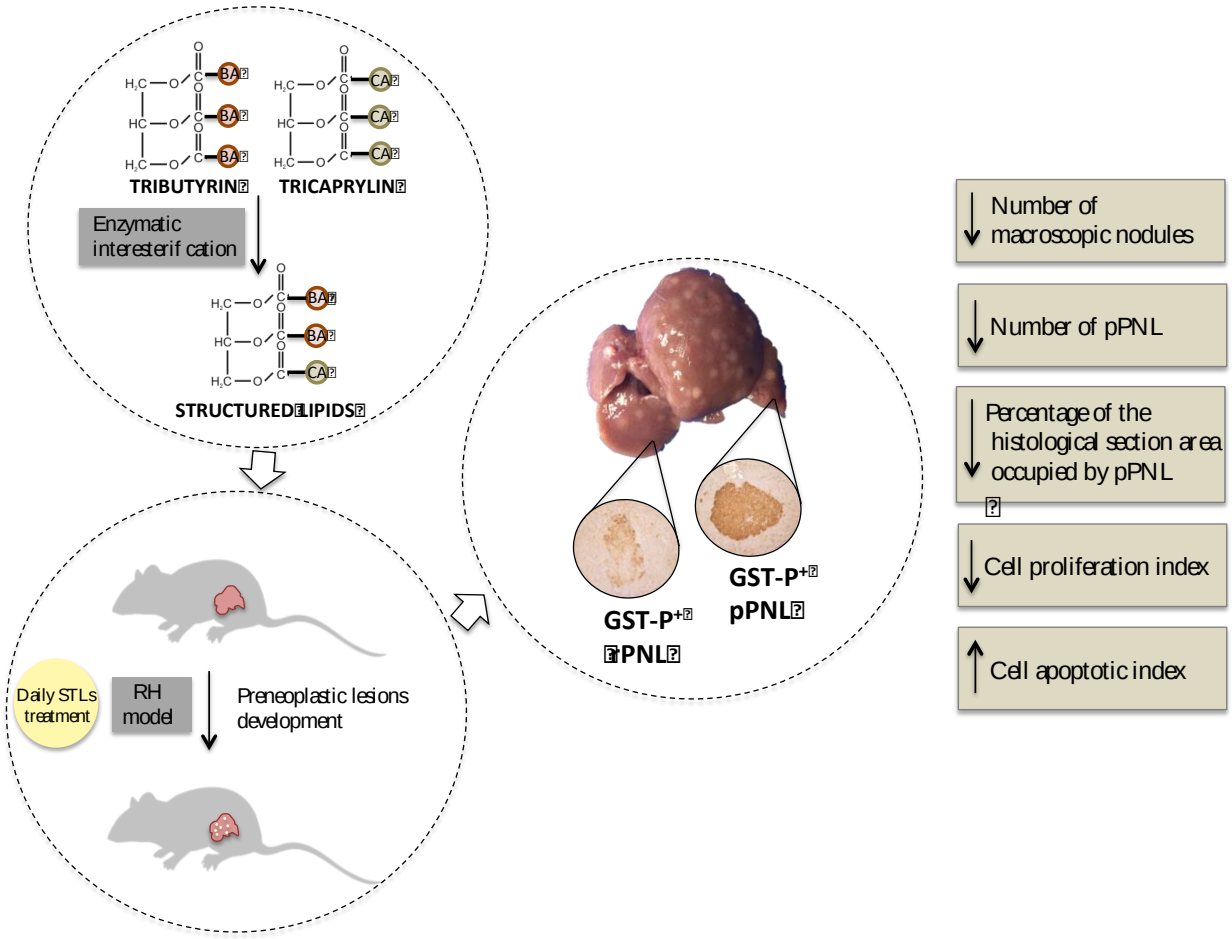
Background and aims: Hepatocellular carcinoma (HCC) chemoprevention has been a field of great interest due to the insufficient treatments and poor prognosis of the disease. Natural compounds may have chemopreventive activity against many types of cancer. Butyric acid (BA), product of dietary fiber fermentation, is a well-established anticarcinogenic agent that acts as a histone deacetylase inhibitor in experimental HCC. However, its unfavorable pharmacokinetic profile limits its clinical application. Caprylic acid (CA) is widely used to enhance the absorption of other compounds. CA acts as a tight junction modulator and may improve the paracellular absorption of BA. In this sense, a drug delivery system based on butyrate carrier structured lipids may consist of a potential chemopreventive strategy against HCC. Thus, this study aimed to produce structured lipids (STLs) from BA and CA source triacylglycerols - tributyrin (TB) and tricaprylin (TC), respectively - and to evaluate their chemopreventive activity during the initiation and promotion phases of hepatocarcinogenesis.
Method: STLs were produced by the enzymatic interesterification of a TB/TC blend. Fischer-344 rats were submitted to the resistant hepatocyte model, which allows the study of the HCC development as it is observed in humans. The animals were treated either with maltodextrin (MD), an isocaloric control, or STLs before and during the preneoplastic lesions (PNLs) development. Liver macroscopic nodules were evaluated, as well glutathione S-transferase (GST-P) positive PNLs. PNLs were classified in persistent (pPNL), which progress to HCC, or remodeling (rPNL), the ones that tend to return to a normal phenotype. PCNA labeling nuclei, as well apoptotic bodies were evaluated in pPNL and rPNL by immunohistochemical and TUNEL assay, respectively. Hepatic concentrations of BA and CA were performed by CG/MS.
Results: Treatment with STLs reduced ($p<0.05$) the number of macroscopic nodules as well as the number of pPNL and the percentage of the histological section area occupied by them, when compared to MD treatment. The STLs group showed lower cell proliferation index ($p <0.05$) and higher apoptotic index ($p=0.05$), than MD group. As expected, STLs group showed higher ($p <0.05$) BA and CA concentrations than the MD group.
Conclusion: Hepatocarcinogenesis chemopreventive activity of STLs is related to their suppressive action in pPNL, the most aggressive type of liver preneoplasic lesion.

Figure:


TRIBUTYRIN
TRICAPRYLIN
Enzymatic interesterif cation
STRUCTURED LIPIDS
Daily STLs treatment
RH model
Preneoplastic lesions development
GST-P+ pPNL
GST-P+ pPNL
Number of macroscopic nodules
Number of pPNL
Percentage of the histological section area occupied by pPNL
Cell proliferation index
Cell apoptotic index

P04-22 Application of serum cytokeratin-19 fragments (CYFRA 21-1) in predicting the prognosis of patients with hepatocellular carcinoma

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Background and aims: Cytokeratin-19 (CK-19) is a cancer stem cell marker expressed by a subpopulation of hepatocellular carcinomas (HCCs) associated with tumor aggressiveness. It has been shown that serum levels of CK-19 fragments (CYFRA 21-1) correlate with the HCC expression of CK-19; hence we evaluated the prognostic value of serum CYFRA 21-1 compared to alpha-fetoprotein (AFP) and protein induced by vitamin-K absence-II (PIVKA-II) in patients with HCC.

Method: A total of 160 patients (28F/132M; age 65 [44 - 86] years) with a new diagnosis of HCC achieved between Nov-2012 and Jan-2018 were retrospectively analyzed. All patients had cirrhosis and the main underlying etiology was viral (118/160, 73.8%). Barcelona Clinic Liver Cancer (BCLC) staging system was adopted for patients classification and therapies allocation (18 stage 0, 77 A, 39 B, 23 C, 3 D). Radiological response to treatment was assessed by modified Response Evaluation Criteria in Solid Tumors (mRECIST). Serum samples were collected at HCC diagnosis; CYFRA 21-1, AFP and PIVKA-II were measured on Lumipulse® G600 II (Fujirebio Inc, Japan).

Results: The median overall survival (OS) after HCC diagnosis was 35.1 (95%CI 27.1 - 35.1) months. At HCC diagnosis, median CYFRA 21-1, AFP and PIVKA-II levels were 1.3 (95%CI 1.2 - 1.5) ng/mL, 12.6 (95%CI 8.6 - 16.9) ng/mL and 199 (95%CI 146 - 316) mAU/mL, respectively. CYFRA-21 showed a poor correlation with BCLC staging (rs = 0.157, p = 0.048) and PIVKA-II (rs = 0.163, p = 0.039) but no correlation with AFP (rs = 0.072, p = 0.368). At univariate analysis, CYFRA 21-1 > 2.7 ng/mL resulted a significant factor for poor prognosis (Log-rank test, p < 0.001). Multivariate Cox regression showed that CYFRA 21-1 > 2.7 ng/mL (HR = 3.45, 95%CI 1.84 - 6.46), AFP > 20 ng/mL (HR = 2.20, 95%CI 1.24 - 3.90), PIVKA-II > 200 mAU/mL (HR = 2.38, 95%CI 1.29 - 4.39), BCLC stage (HR = 1.61, 95%CI 1.20 - 2.16) and radiological response (HR = 0.21, 95%CI 0.08 - 0.55) were independent predictors of 3-year OS, while only CYFRA 21-1 > 2.7 ng/mL (HR = 3.46, 95%CI 1.69 - 7.10), BCLC stage (HR = 2.00, 95%CI 1.36 - 2.95) and radiological response (HR = 0.09, 95%CI 0.01 - 0.72) were independent predictors of 1-year OS.

Conclusion: Among investigated biomarkers, CYFRA 21-1 resulted the strongest independent predictor of OS. The determination of baseline serum CYFRA 21-1 may be a useful prognostic factor.

P05-01YI Hepatocellular carcinoma in non-cirrhotic liver arises in a more advanced tumoral appearance

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Background and aims: Hepatocellular Carcinoma (HCC) usually arises in a cirrhotic liver (CL) and a small proportion is detected in the non-cirrhotic liver (NCL). However, little is known about the characteristics and prognosis of HCC in a NCL. The present study aimed to compare the characteristics and prognosis of HCC in NCL and CL.

Method: A retrospective comparison analysis was performed in a cohort of 41 patients with HCC in NCL and 321 patients with HCC in CL with regard to demographic, laboratory, clinical and tumoral characteristics recorded at the time of diagnosis. The Mittal criteria were constructed to increase confidence in the cirrhotic or non-cirrhotic status assignment of the subgroup of patients who lacked liver histology for confirmation. Survival analysis was conducted using Kaplan-Meier Method, and the survival probability were compared using the Log-rank test.

Results: Etiologies of NCL-HCC group were consisted of 26 (63.4) HBV, 10 (24.4) NAFLD/cryptogenic and 5 (12.2) HCV patients, which was similar to the distribution of HCC etiologies in CL. Median maximum tumor size was higher in NCL-HCC group (90 (16-200) mm vs. 46 (8-190) mm, p<0.001). Vascular invasion and extra-hepatic metastasis were more common in NCL-HCC group (11 (26.8%) vs 23 (7.3%), p=0.001, and 6 (14.6%) vs 10 (3.1%), p=0.005, respectively). NCL-HCCs were less prone to curative options according to Milan Criteria (9 (22%) vs 140 (43.2%), p=0.006). The median survival probabilities were comparable among HCCs in NCL and CL (14.76 (1.75-27.78) vs. 17.53 (12.78-22.28), p=0.753).

Conclusion: Patients with HCC in NCL appears in larger size, and spreading to extrahepatic veins and organs. Despite these differences, survival is similar among HCCs in NCL and CL. The optimized surveillance programs for those without cirrhosis and carrying high risk for HCC development may improve the features of HCC in NCL.

Figure:

Table 1. Demographics and characteristics of HCC patients in non-cirrhotic and cirrhotic liver

	Non-cirrhotic (n=41)	Cirrhotic (n=321)	p value
Age, *median (IQR), years*	66 (23-89)	67 (33-90)	0.756
Male gender, n(%)	31 (75.6)	242 (74.7)	0.535
BMI, mean±SD, kg/m^2	27.2±4.2	27.7±5.1	0.645
Obesity, n(%)	6 (21.4)	49 (27.7)	0.329
DM, n(%)	11 (26.8)	116 (35.8)	0.168
HT, n(%)	16 (39)	102 (31.5)	0.211
HL, n(%)	10 (24.4)	45 (13.9)	0.067
Cigarette, n(%)	16 (39)	112 (34.6)	0.344
Alcohol, n(%)	8 (19.5)	54 (16.7)	0.849
Etiology, n(%)			
- HBV	26 (63.4)	179 (55.3)	
- NASH/cryptogenic	10 (24.4)	78 (24.1)	
- HCV	5 (12.2)	56 (17.3)	0.937
- Etilism	-	7 (2.2)	
- Autoimmune	-	3 (0.9)	
- Other	-	1 (0.3)	
AST, *median (IQR), U/L*	61 (17-636)	53 (13-365)	0.287
ALT, *median (IQR), U/L*	42 (11-426)	41 (7-727)	0.944
T.bil, *median (IQR), mg/dl*	0.83 (0.4-2)	1.2 (0.2-12.8)	<0.001*
Alb, *median (IQR), gr/dl*	4 (3.6-4.8)	3.56 (1.7-8)	<0.001*
Plt, *median (IQR), x1000/m^3*	227 (141-441)	122 (28-838)	<0.001*
INR, *median (IQR)*	1.1 (0.89-1.51)	1.23 (0.84-3.9)	<0.001*
AFP, *median (IQR), ng/ml*	11.8 (1.58-38630)	15.4 (1.26-300000)	0.326
ECOG, n(%)			
- **0**	34 (82.9)	210 (66.5)	
- **1**	5 (12.2)	64 (20.3)	0.279
- **≥2**	2 (4.9)	42 (13.2)	
Max tm. size, *median (IQR), mm*	90 (16-200)	46 (8-190)	<0.001*
Number of lesions, n (%)			
- 1	26 (63.4)	194 (59.9)	
- 2	5 (12.2)	46 (14.2)	
- 3	2 (4.9)	27 (8.3)	0.737
- multiple	8 (19.5)	50 (15.4)	
- diffuse	-	7(2.2)	
BCLC, n(%)			
- 0-A	7 (17.1)	113 (35.2)	
- B	18 (43.9)	106 (32.7)	0.029*
- C	16 (39)	81 (25)	
- D	-	24 (7.4)	
Lymph node, n(%)	7 (17.1)	63 (20)	0.420
Vascular invasion, n(%)	11 (26.8)	23 (7.3)	0.001*
Metastasis, n(%)	6 (14.6)	10 (3.1)	0.005*
Milan criteria fulfillment, n(%)	9 (22)	140 (43.2)	0.006*
Treatment, n(%)			
- Curative options	7 (17.1)	87 (27.1)	
- Non-curative options	30 (73.2)	194 (60.4)	0.039*
- BSC	4 (9.7)	40 (12.%)	

P05-02 Global characterisation of tumor infiltrate of intrahepatic cholangiocarcinoma by single-cell sequencing

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Background and aims: Intrahepatic cholangiocarcinoma (iCCA) is the second most common primary liver cancer, characterized by high resistance to chemotherapy and poor prognosis. We have previously demonstrated that the tumor immune microenvironment (TME) has a prognostic impact on iCCA patients; however, little insight exists on immune subsets involved in iCCA and precise criteria to assess tumor biology are still lacking.

Method: we examined immune infiltrate with single cell-RNA sequencing (scRNAseq) of iCCA tumor and peritumor liver sample. scRNAseq was performed on CD45+ sorted cells isolated from tumoral and peritumoral sample of iCCA patients (n=6) surgically resected at the Division of Hepatobiliary and General Surgery in Humanitas. Cell suspensions were converted to barcoded scRNAseq libraries with 10x Genomics Chromium Single-cell system and were sequenced on Illumina NextSeq 500. CellRanger (v3.0.1, 10x Genomics) pipeline were applied to obtain gene espression data. For each cluster, gene average expression and marker genes were obtained. A principal component analysis (PCA) was performed both for the whole dataset and for each cluster of cells. Clusters classification by cell types was performed by comparing each single cell gene expression with public transcriptomic datasets of pure cell types by using the SingleR (v0.9) Bioconductor R package.

Results: We obtained an integrated dataset of 12 samples for a total of more than 30,000 good quality single cells with a median of 800 detected genes each. This analysis revealed that tumor samples clearly separate from peritumoral ones according to the main principal immune cells population for each patient. Key actor of these differences, identified by clustering and classification analysis, were T cells, NK cells and myeloid cells. Moreover, each of them is characterized by cell subpopulations with transcriptional differences between tumoral and peritumoral samples.

Conclusion: These results highlighted that TME strongly differs from the immune system infiltrating the peritumoral area. Further, our study provides a new approach for patient stratification and will help further understand the functional states and dynamics of TME in iCCA.

P05-03YI Filamin-A expression predicts recurrence of mass-forming cholangiocarcinoma after hepatectomy

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Background and aims: Recurrence of mass-forming cholangiocarcinoma (MFCCC) after hepatectomy is very high. A predictive marker of recurrence capable of personalizing follow-up and developing new targeted therapy would be beneficial. The overexpression of Filamin-A (FlnA), a cytoskeleton protein with scaffolding properties, has recently been associated with cell signalling, migration and adhesion in different tumors. The aim of this study was to test the expression of FlnA in a cohort of patients operated for MFCCC.
Method: A retrospective cohort of patients who underwent hepatic resection for MFCCC at Humanitas Clinical and Research Center between January 2004 and December 2018 was analyzed. FlnA expression was measured by calculating its intensity score at immunohistochemistry on paraffin-embedded tumor tissue sections for each patient. Such expression was then correlated with prognostic parameter of disease-free survival (DFS) by using survival analyses.
Results: A total of 82 patients were considered. Median DFS in patients with low expression of FlnA was significantly increased in comparison with patients with high expression of FlnA (27 months vs. 10 months). Similarly, 5-year DFS was 30.8% vs. 10.9% (P=0.008). At the multivariate analysis number of tumor (HR=2.18; CI95% 1.98-3-21; P=0.004), tumor grade (HR=2.81; CI95% 1.77-5.12; P=0.001) and high expression of FlnA (HR=1.81; CI95% 0.98-2.31; P=.0.005) were found to be independently associated with worse DFS.
Conclusion: FlnA expression is associated with higher risk of recurrence of MFCCC after hepatectomy. This finding provides important insights that would help physicians to personalize follow-up strategies and develop targeted therapy.

Figure:

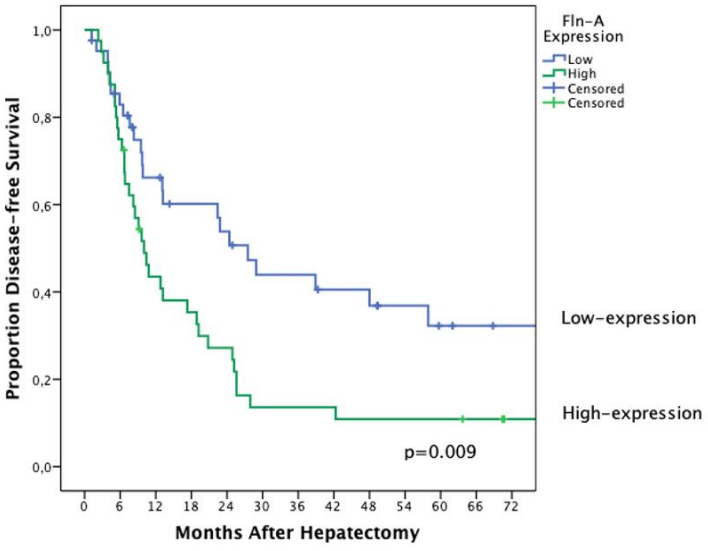

Fig.1: FlnA expression related to patients DFS: low expression of FlnA correlates with lower disease recurrence.

P05-04YI In vivo performance of PEG-coated gold nanoparticles mediated ultrasound guided radiofrequency ablation: A pilot study in swine

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Background and aims: Radiofrequency ablation (RFA) is the first line treatment option for patients with small hepatocellular carcinoma and a recommended treatment modality for patients with hepatic metastases judged to be unfit for surgical intervention. However, RFA is still used mainly for lesions < 3 cm, and despite all great advancements the ablation zone remains limited in volume. Gold nanoparticles offer the potential to heat tumour tissue selectively at the cellular level by non-invasive interaction with radiofrequency energy delivered externally. The objective of this study was to investigate the effects of polyethylene glycocol (PEG) coated gold nanoparticles intratumorally delivery on ablation zone volumes during in vivo RFA of porcine liver.

Method: This prospective study was performed following institutional animal care and use committee approval. RFA was performed in liver in ten Sus scrofa domesticus swine. PEG coated gold nanoparticles (10 millilitres of solution for each ablation with a concentrations of $1x10^{11}$ and $1x10^{7}$ nanoparticles/ml) were injected through the radiofrequency antennae during ablation. Physiological saline (10 millilitres for each ablation) was used as control. Ten minutes after ablation contrast enhanced ultrasound (CEUS) was performed to evaluate the necrosis volume. Animals were kept under close medical observations for 5 days. On day 5 another CEUS examination was performed and animals were using a sodium pentobarbital solution. Treated tissues were explanted and the necrosis volume was measured using calipers. Hematoxylin and eosin (H&E) staining was also performed for histological analysis.

Results: A total of 30 ablations were performed in the liver. The use of PEG coated gold nanoparticles significantly increased ablation zone volumes (13.61 cm^3 versus 5.26 cm^3, $p < 0.001$). The increase of ablation zone volumes was not dose dependently (13.61 cm^3 versus 11.84 cm^3, $p = 0.17$). There was no difference between ablation zone volumes assessed by CEUS immediately after ablation compared to CEUS performed 5 days after ablation ($p = 0.34$). H&E staining showed no differences in the transition zone (the zone between coagulative necrosis and normal parenchyma) between physiological saline or nanoparticles enhanced RFA.

Conclusion: The use of PEG coated gold nanoparticles significantly increased mean ablation zone volumes following direct intratumoral RFA in a porcine model. To our knowledge this is the first study dealing with direct heating of PEG-coated nanoparticles by RF energy. However, the positive finding from our study warrants further investigations.

P05-05YI New prognostic score utilizing glypican-3 serum levels for the prediction of 6-month outcome after transarterial therapies for patients with intermediate stage hepatocellular carcinoma

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Background and aims:
The selection of patients with intermediate stage hepatocellular carcinoma (HCC) for transarterial therapies currently relies on liver function and tumor properties. Outcome prediction by circulating biomarker is desirable, but not established. The aim of this study was to assess experimental HCC biomarkers for their association with survival after transarterial therapies.
Method:
Data were retrospectively collected from 125 consecutive HCC patients with intermediate stage HCC (BCLC-B) undergoing either transarterial chemoembolization (TACE, n = 98) and/ or selective internal radiation therapy (SIRT, n = 28) at one German University Hospital between 2010 and 2017. Patients and tumor characteristics including total diameter and number of tumor lesions, C-reactive protein (CRP), platelets, leucocyte counts, albumin, bilirubin and alanine transaminase (ALT) levels as well as circulating HCC biomarkers including AFP, AFP-L3, des-gamma-carboxy prothrombin (DCP), alone and summarized in the GALAD score, glypican-3 (GPC-3), and Dickkopf-related protein 1 (Dkk-1) were quantified on cryopreserved serum samples taken prior to therapy. Univariate and multivariate analyses were performed to identify the predictive value of the different biomarkers for 6-month survival. The parameters with the strongest independent association with survival were included into a new scoring system.
Results:
Univariate analyses showed that lower GPC-3 as well as DCP serum levels were significantly associated with 6-month survival (median GPC-3: 8 pg/ml vs. 32 pg/ml, p = 0.002; DCP: 4 ng/ml vs. 16 ng/ml, p = 0.004). Moreover, higher GALAD-scores were associated with a poorer prognosis (4.85 vs. 1.32, p = 0.008). In contrast, all other patient and tumor related parameters showed no significant difference regarding 6-month survival.
By multivariate analysis, the biomarkers with the highest association with treatment response were GPC-3, the number of lesions and CRP. A combination of the three markers in a new prognostic score GLC (GLC = 2.873 - 2.161/100 * **G**PC-3 (pg/ml) - 0.185 * number of **l**esions - 0.052 * **C**RP (mg/l))was developed resulting in a specificity of 72.7% and sensitivity of 77.9% for prediction of 6 months survival (Fig. 1).
Conclusion:
The combination of novel HCC biomarkers might be a potent tool for tailoring individualized treatment strategies for patients with intermediate stage HCC. The GLC-Score can help to estimate the 6-month-survival rate of these patients with high sensitivity and specificity. A GLC-Score above 1.4 is associated with a 6-month survival rate of 80%.

Figure:

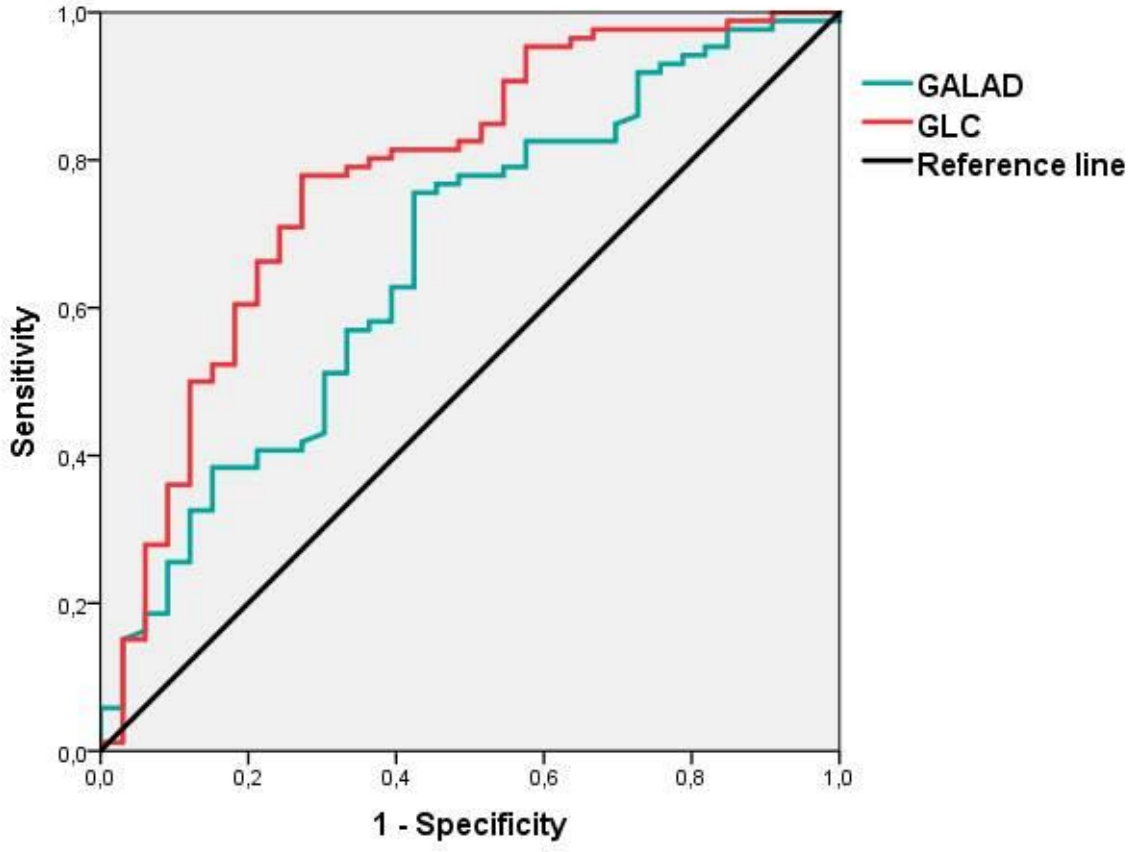

Fig 1: Receiver operating characteristic (ROC) analysis of sensitivity and specificity by the GALAD and the GLC score in predicting 6-month-survival.

P05-06 Distinct mechanisms are responsible for the activation of the NRF2-KEAP1 pathway at different steps of hepatocarcinogenesis

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Background and aims: The Nrf2-Keap1 pathway represents the main intracellular defence against environmental stress and its activation has been observed in several human cancers, including hepatocellular carcinoma (HCC). Different molecular mechanisms, such as Nrf2/Keap1 gene mutation, methylation-induced gene silencing or Keap1 sequestration, p62 accumulation, and consequently Nrf2 nuclear translocation, have been shown to be involved in the activation of this pathway.
Method: A nutritional model of rat hepatocarcinogenesis consisting of a single dose of diethylnitrosamine (DENA) followed by a choline-methionine-deficient (CMD) diet for 4 months was used. CMD diet was then replaced with basal diet and the animals were sacrificed after 6,10 and 13 months from DENA injection. Activation of Nrf2/Keap1 pathway was evaluated by qRT-PCR. Immunohistochemistry and immunoprecipitation. Gene sequencing was performed on Laser Capture-Microdissected lesions.
Results: Nrf2-Keap1 pathway activation was detected since the early steps of hepatocarcinogenesis. While Nrf2 mutations were extremely frequent at early steps of hepatocarcinogenesis (90%), their number diminished with the progression to malignancy (25%). Interestingly, while p62 levels were almost undetectable in early preneoplastic nodules, accumulation of p62 and Keap1 occurred in HCCs suggesting that activation of Nrf2 at late stages of the process could be a consequence of Keap1 sequestration by p62 and Nrf2 nuclear translocation. Indeed, immunoprecipitation demonstrated binding of p62 to Keap1 only in HCCs devoid of Nrf2 mutation and not in preneoplastic nodules carrying gene mutation
Conclusion: These results demonstrate that in a nutritional model of hepatocarcinogenesis, resembling human non-alcoholic fatty liver disease, Nrf2 mutations are the earliest molecular changes responsible for the activation of the Nrf2-Keap1 pathway. However, their progressive loss during HCC development paralleled by accumulation of p62 at late stages implies that non-mutational events are also responsible for the activation of the Nrf2-Keap1 pathway.

P05-07YI Prognostic role of blood eosinophil count in sorafenib-treated hepatocellular carcinoma patients: time to reconsider the minorities

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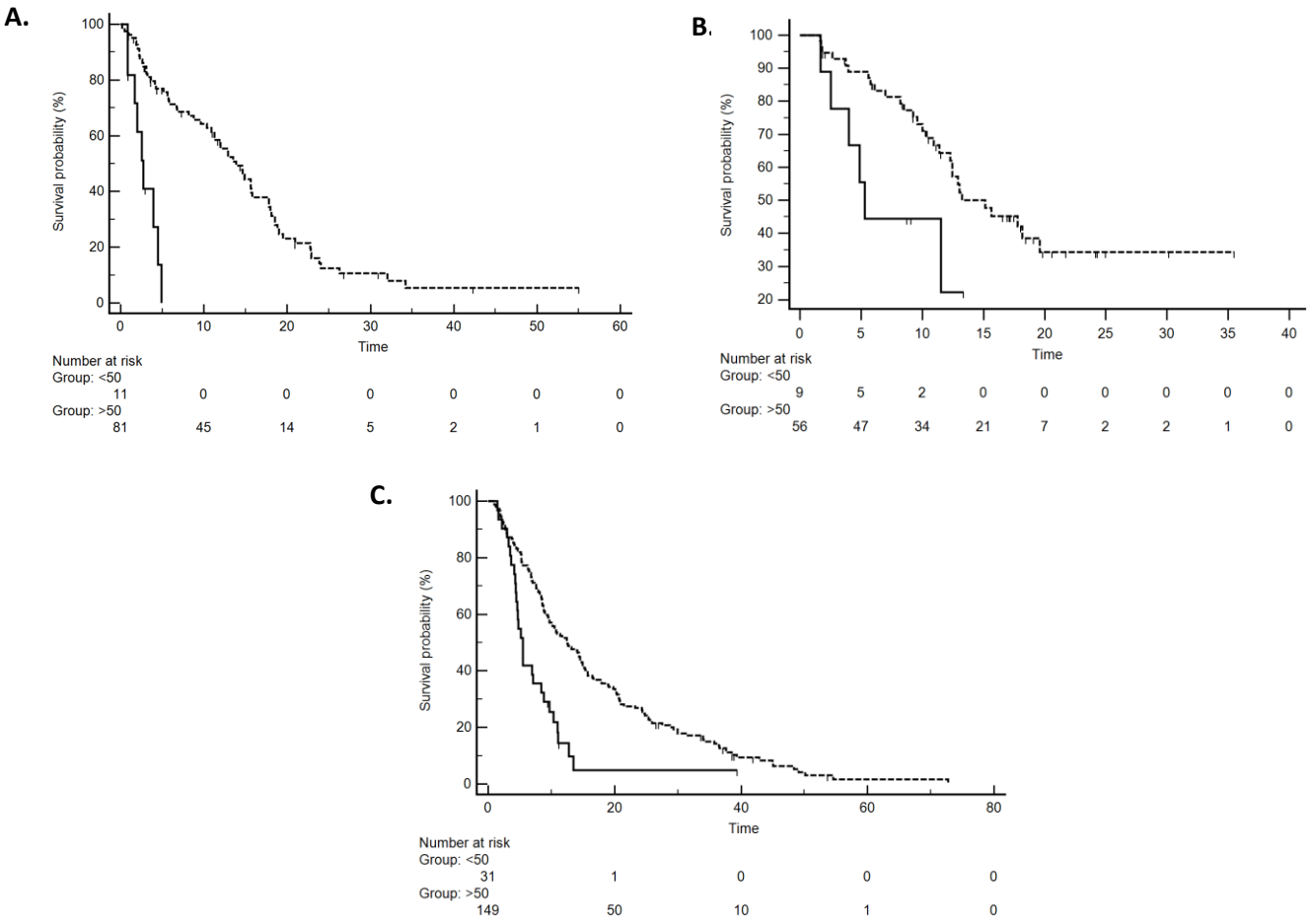
Background and aims: Inflammation is a long-established hallmark of liver fibrosis and carcinogenesis. Eosinophils are emerging as crucial components of the inflammatory process influencing the development of cancer. In the present study we analyzed the prognostic role of baseline blood eosinophil count in Sorafenib-treated Hepatocellular Carcinoma (HCC) patients.

Method: A training cohort of 92 advanced- or intermediate-stage HCC patients treated with Sorafenib from 2007 to 2018 and two independent validation cohorts of 65 and 180 patients were considered. Overall Survival (OS) and Progression-Free Survival (PFS) in relation to baseline eosinophils (≤ or ≥ 50 10^9/L) were estimated by the Kaplan-Meier method and curves were compared by the log-rank test. In addition, univariate and multivariate analyses were performed.

Results: A negative prognostic impact of low baseline eosinophils (<50 10^9/L) was demonstrated in all the cohorts examined [Training cohort: HR for OS= 7.56, 95% CI 2.05–27.93, p=0.0024 for low vs high eosinophils; first validation cohort: HR for OS =4.55, 95% CI 1.24–16.65, p=0.022; second validation cohort: HR for OS =3.21, 95% CI 1.83–5.64, p<0.0001] (Figure 1). Moreover, eosinophil count was the only clinical covariate retaining a prognostic value for OS at multivariate analysis across the whole study population. In addition, for patients who progressed on Sorafenib and received either second-line treatment (capecitabine or Regorafenib) or best supportive care (BSC), low eosinophils had a negative prognostic role only in those treated with Regorafenib and not with capecitabine or BSC.

Conclusion: Our analysis identified baseline blood eosinophils as a new prognostic factor in HCC patients receiving Sorafenib. Moreover, eosinophils were associated with survival outcomes only in patients receiving Regorafenib as second-line treatment, suggesting a possible predictive role in this setting.

Figure:
Figure 1: Kaplan-Meier curves for OS in the training cohort (A), in the first (B) and in the second (C) validation cohorts.



P05-08YI Prevalence and impact of sarcopenia in patients undergoing liver transplantation for hepatocellular carcinoma

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Background and aims: Sarcopenia is associated with poor outcomes in patients with cirrhosis and hepatocellular carcinoma (HCC) as well as with poor liver transplantation outcomes. The aim of the present study was to assess prevalence of sarcopenia and its impact on survival and HCC recurrence after liver transplantation (LT).

Method: We retrospectively analysed 72 HCC patients who underwent LT from 2013 to 2018. Sarcopenia was defined when the height-normalized psoas muscle thickness was <16.8 mm/m at the L3 vertebra level on computed tomography scan that was performed within 6 months prior to LT. HCC assessment was based on surgical specimen.

Results: Patients were predominantly males (83.3%) with mean age 62.56±6.34 years at the time of LT. Majority of patients (50%) had underlying alcohol-related liver disease, following hepatitis C infection (26%). Overall, 63 (87.5%) patients met definition of sarcopenia. Patients without sarcopenia had significantly higher body mass index (30.72±4.97 vs 26.71±6.70, p=0.04). On the other hand, there was no significant difference in the prevalence of metabolic syndrome, arterial hypertension, diabetes mellitus, cholesterol and triglycerides serum levels between sarcopenia and non-sarcopenia group. A group without sarcopenia had numerically reduced HCC burden defined as lower average AFP level (1210±1898 vs 1351±555), number of lesions (2.56±2.35 vs 4.42±3.63), sum of all tumour size in mm (54±28.18 vs 116.99±84.11), micro- and macro-vascular invasion (38 vs 46 %, and 8 vs 7 %, respectively), although none of the above mentioned reached statistical significance. Overall, 46.67% patients were beyond Milan criteria without difference between sarcopenia and non-sarcopenia group. There were no significant difference in HCC recurrence (22.22% vs 14.29 %) or survival (66.67% vs 76.18%) between groups during average follow-up of 2.65±1.66 years.

Conclusion: The most important findings of presented research are high prevalence of sarcopenia among HCC patients undergoing LT and high prevalence of patients who were beyond Milan criteria based on surgical specimen. These results are contrary to previously published data that defined sarcopenia as negative predictive factor for death and HCC recurrence, although not in the same patient population.

P05-09 Dissecting a novel strategy to target mitochondria in bile duct cancers

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Background and aims: Mitochondria (Mi) are fundamentally implicated in cancer biology, including initiation, growth, metastasis, relapse, and acquired drug resistance. Mi are considered as the target organelles for therapeutic strategies of several cancers including CCA. This project aims to provide a novel approach targeting Mi in CCA, by elucidating at molecular level a novel Mi-related cell death pathway, recently identified by our group, who first showed that genetic alterations in HCCS and COX7B, two components of the mitochondrial respiratory chain, cause apoptosis induced by APAF1-independent CASP9 activation(1). The project aims to dissect the the novel MiPa, to identify all molecular players, to functional characterize their role in CCA, to identify novel targeted therapies, to develop a gene signature exhibiting consistent prognostic power and predictive value as potential biomarker.

Method: HTS has been performed by using Prestwick Chemical Library (Prestwick). The Human siGENOME Druggable-Genome-Library (Dharmacon) has been used in functional experiments. CASP9 activity has measured using the ApoAlert CASP9 assay (Clontech). Cell viability was monitored by CellTiter-Blue assay (Promega). The patient cohort consists of 100 patients retrospectively identified by Dept of Oncology (Univeristy of Modena). Lentiviral-based shRNA and CRISPR/Cas9 systems were utilized to establish stable gene knockdown and knockout model. FFPE-RNA extraction, RNAseq and analyses were performed using protocols available at TIGEM(2).

Results: Molecular players of the novel MiPa, which triggers APAF1-independent caspase-9 activation have been identified using a proteomic approach combined to siRNA library screening. HTS allowed to identify compounds targeting the novel MiPa. Two compounds were found to inhibit tumour growth *in vitro* and their anti-tumour efficacy has been assessed. Experiments in HuCCT-1, KMCH, CCLP, SW1, EGI, TFK1 cell lines revealed their effect on autophagy inhibition and apoptosis and correlated the MiPa pathway with drug treatment response and drug resistance. Whole-transcription sequencing experiments are ongoing to profile the novel MiPa gene signature in cell lines treated and not with standard therapeutic approach, and in a cohort of 100 CCA patients.

Conclusion: A novel MiPa has been identified regulating the apoptosis in CCA. Implication of the novel MiPa in drug resistance and sensitivity to classical therapeutic treatment has been assessed. Two compounds were identified to target the novel MiPa. Whole-transcriptome analysis are ongoing to evaluate the clinical value of the MiPa gene signature in CCA patients.

References

1.Indrieri, A., et al., EMBO Mol Med, 2013. 5(2): p. 280-93.

2.Cacchiarelli D, et al., Cell. 2015 Jul 16;162(2):412-424.

P05-10 Epidemiology, features and outcome of patients transplanted for hepatocellular carcinoma in the last decade: A single center experience

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Background and aims: Hepatocellular carcinoma (HCC) represents an increasing indication for liver transplantation (LT) world-wide but the burden and clinical implications of post-transplant HCC recurrence is still debated. Aim of this study was to identify recurrence rate, survival and predictors of recurrence in HCC patients consecutively transplanted in the last 10-years.

Method: retrospective, single center study including all consecutive LT patients with HCC from 01/2010 to 07/2019. HCC-recurrence surveillance was performed with CT-scan and AFP every 6 months for the first 5-yrs after LT. Immunosuppression was CNI-based.

Results: 182 HCC out of 449 transplanted patients (41%) were studied: 84% males, median age 58-yrs, 60% HCV, 25% MELD≥15, median AFP at LT 9 ng/ml; median time-lag between HCC diagnosis and LT was 17 months, pre-LT bridging/down-staging therapy in 74%. At explant pathology: 16% showed micro-satellitosis, 27% microvascular invasion (mVI), 50% Edmonson score=G3/4; 76% were “Milan”-in, 11% were "Milan"-in and "Up to 7"-in while 13% “Up to 7”-out. During a median follow-up of 42 months, HCC recurred in 29 patients (16%) after a median time of 9 (2-46) months. Probability of recurrence at 1-, 3-, 5-yrs was 5%, 11% and 15%, respectively. By multivariate analysis, independent risk-factors for HCC recurrence were micro-satellitosis (HR=0.31, p=0.023) and microvascular invasion (HR=0.38, p=0.04). Overall probability of survival at 1-, 3- and 5-yrs was 94%, 87% and 77%, respectively; being 95%, 89% and 85% in the recurrence-free patients vs 89%, 76% and 43% in the recurrence-group (p<0.005). Those patients who were transplanted after 2015 (n=108), significantly differed from those transplanted before 2015 (n=74), since they were older (p=0.04), more frequently males (p=0.02), with lower MELD (p=0.0001) at transplant, more frequently treated by locoregional therapies for bridging/down-staging purposes (p=0.002) and with higher rates of G3/4-HCC (p=0.001) at explant pathology, however with similar post-transplant overall survival [100.9 (95%CI 90.16-111.8) vs 91.4 81.7-101.1) months (p=0,15)].

Conclusion: Our study confirms tumor-related features at explant pathology as predictors of HCC recurrence. Moreover, in the last few years we have transplanted older patients with less severe disease but with more advanced tumors, while maintaining survival figures well fitted with a transplant benefit.

P05-11YI Autoimmunity associated with primary biliray cholangitis prevents the development of cholangiocarcinoma in mouse models

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Background and aims: Primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are two chronic inflammatory diseases of the biliary tract. Yet, chronic inflammation is known to be one of the main risk factors for cancer onset. In this line, PSC is the first etiology of cholangiocarcinoma (CCA). But surprisingly, patients with PBC never develop CCA. Therefore, we hypothesised that PBC-associated autoimmunity could prevent CCA appearance.

Method: To assess whether or not PBC could protect from CCA onset, we adapted two models of cholangitides in mice and obtained CCA cell lines syngeneic in immunocompetent mice. PBC, PSC and control mice were challenged subcutaneously with CCA cells (or with irrelevant control fibrosarcoma or lung carcinoma cells) and tumor growth was monitored. To assess the role of the lymphocyte subsets in vivo, we performed selective depletion of CD4+ and/or CD8+ T cells, or B cells through injections of depleting antibodies. Secondarily, the involvement of type 1 or 17-polarized T cells was evaluated in vivo with specific neutralization of interferon gamma (IFNg) and interleukin 17 (IL-17) using antibodies. Immune gene signature in the liver of PBC, PSC, and healthy livers were compared at the transcriptomic level. Finally splenocytes from PBC mice, PSC mice and healthy control were collected after cholangitis induction and co-cultured with CCA cells. Then, the presence of CCA-specific cognate T cells was studied by detecting intracellular cytokine production by flow cytometry.

Results: We observed a significant delay in CCA growth in PBC mice compared with control and PSC mice. These data validate our hypothesis by demonstrating that PBC-associated autoimmunity protects against CCA. PBC-mediated CCA immunosurveillance was mainly depending on T lymphocytes, with a contribution of both CD4+ and CD8+ T cell subsets. Moreover, in vivo blockade of either IFNg or IL-17 impaired the anti-CCA immune response upon PBC. This anti-tumor activity was specific of CCA as PBC animals were not protected against the outgrowth of non biliary tumors. Finally, ex vivo experiments allowed to identify IL-17 as the major cytokine produced by PBC-associated CD4+ and CD8+ T splenocytes upon exposure to CCA cells.

Conclusion: In mouse models of cholangitides, we demonstrated that the autoimmunity associated with PBC could specifically prevent CCA development.

P05-12YI Mitochondrial oxidative metabolism contributes to maintain a cancer stem cell phenotype in cholangiocarcinoma

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Background and aims: Accumulating evidence indicates cancer stem cells (CSC) as a key target in cancer. Although metabolic reprogramming is considered an important feature of cancer cells, little is known about metabolic regulation in CSC derived from cholangiocarcinoma (CCA). This study investigated the role of mitochondria-dependent metabolism and of the related signaling pathways in the maintenance of a stem-state in CCA.

Method: Stem-like subset was enriched by sphere culture (SPH) in established human intrahepatic CCA cells (HUCCT1, CCLP1). Extracellular flux analysis was examined by Seahorse technology. Mitochondrial membrane potential and mitochondrial mass were assessed by MitoTracker Red and MitoTracker Green, respectively. Glucose uptake was quantified by incorporation of (U-14C)-D-Glucose. Gene set enrichment analysis (GSEA) and correlation with overall survival (OS) (log rank/Mantel-cox statistics) and time to recurrence (TTR) (Gehan-Breslow Wilcoxon test) were carried out from a transcriptome database of 104 CCA patients.

Results: In contrast to parental cells grown as adherent monolayers (MON), metabolic analyses by Seahorse revealed a more efficient respiratory phenotype in CCA-SPH, due to mitochondrial oxidative phosphorylation. In addition, CCA-SPH retained high mitochondrial membrane potential and elevated mitochondrial mass, as well as over-expression of PGC-1α, a master regulator of mitochondrial biogenesis. In vitro targeting of mitochondrial complex I by metformin impaired the ability to form SPH, expression of CSC-associated genes, and genes related to pluripotency and epithelial mesenchymal transition. In an in vivo model in immunocompromised mice, growth of tumors derived from CCA-SPH was suppressed by metformin. Furthermore, PGC-1α silencing highly reduced the expression of stem-like markers in CCA-SPH, and reduced sphere-formation and cell invasion. Notably, GSEA analysis showed that patients with with high levels of mitochondrial complex II had a worse prognosis in terms of OS (p=0.036) and TTR (p=0.029). In addition, PGC-1α was significantly correlated with mitochondrial complex II and stem-like genes in CCA patients.

Conclusion: Our data indicate a pivotal role of mitochondrial oxidative metabolism in the biology of the stem-like subset in CCA.

P05-13 Association between survival of patients with hepatocellular carcinoma and [18F]-fluoro-2-deoxy-D-galactose positron emission tomography/computed tomography

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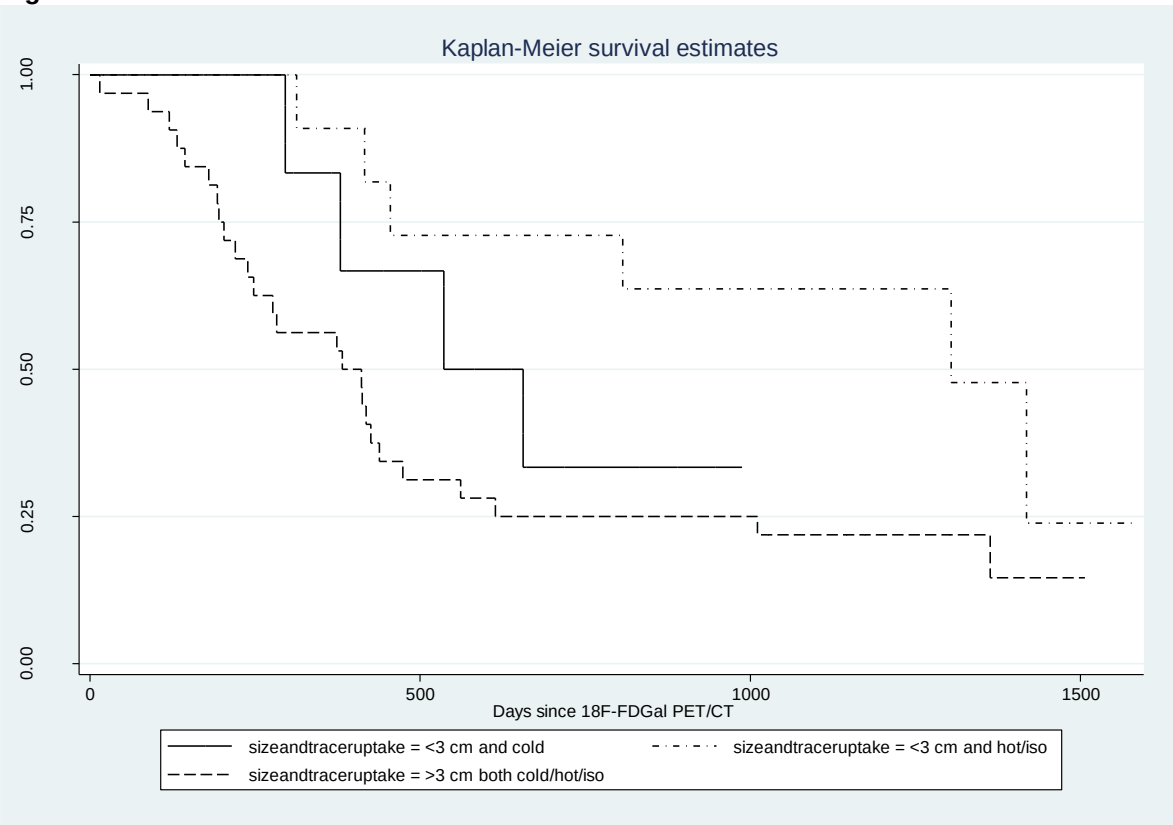
Background and aims: [^{18}F]-Fluoro-2-deoxy-*D*-galactose (^{18}F-FDGal) is a liver specific tracer for positron emission tomography/computed tomography (PET/CT) that is useful for detection of extrahepatic HCC metastases. We tested the hypothesis, that the uptake pattern in intrahepatic HCC foci of ^{18}F-FDGal is related to survival.

Method: Survival data, sex, Child-Pugh class (CP), WHO performance status (PS) were collected along with information on ^{18}F-FDGal uptake patterns and size of intrahepatic HCC from 49 patients included in a recently published cohort (Liver International 2019, in press) who underwent ^{18}F-FDGal PET/CT during November 2014 through March 2017. Patients were followed until death or medio November 2019. Survival was examined by Kaplan-Meier curve analysis and tested for identical incidence with log-rank test. For patients with more than one intrahepatic focus, the uptake pattern in the largest foci were used for analysis. Uptake pattern was scored as same or higher (iso/hot) than surrounding liver tissue or lower (cold).

Results: Thirteen patients (27%) were alive at medio November 2019. The overall median survival was 478 days (95% CI 379;656) with no significant difference between newly diagnosed or patients with recurrence (P =0.99) or sex (P =0.34). Median survival for patients without cirrhosis was 614 days vs 379 days for patients with cirrhosis (P=0.03), 614 days for patients with PS 0 vs 248 days for patients with PS 1-2 (P=0.004), and 1304 days for patients with foci <3 cm vs 382 days for patients with >3 cm (P = 0.02). Median survival for patients with cold foci was 411 days vs. 807 days for patients with iso/hot foci (P = 0.19). Median survival for patients with small, hot/iso foci was 1304 days vs 536 days for small, cold foci and 382 for large foci (P=0.045).

Conclusion: Patients with intrahepatic HCC foci <3 cm and uptake of ^{18}F-FDGal equal to or higher than surrounding liver tissue was associated with a better prognosis than small foci with low uptake and large foci regardless of uptake. This finding may be related to tumour differentiation, with well and medium differentiated tumours having an uptake pattern more similar to hepatocytes and low-uptake foci being poorly differentiated.

Figure:


Kaplan-Meier survival estimates
1.00
0.75
0.50
0.25
0.00
0
500
1000
1500
Days since 18F-FDGal PET/CT
sizeandtraceruptake = <3 cm and cold
sizeandtraceruptake = <3 cm and hot/iso
sizeandtraceruptake = >3 cm both cold/hot/iso

P05-14YI Endothelial inducible T cell co-stimulator ligand (ICOSL) regulates adhesion molecule expression on liver endothelium: implications for senescence-mediated immune surveillance

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Background and aims: Clearance of precancerous cells from the liver is mediated via an inherent tumour suppressor mechanism known as oncogene-induced senescence (OIS). Cells undergoing OIS drive this autonomous deletion by the release of a proinflammatory cocktail, known as the senescence-associated secretory phenotype (SASP). OIS cell clearance from the liver is dependent on liver-infiltrating $CD4^+$ T cells and we have previously shown that the SASP drives lymphocyte recruitment to primary human liver sinusoidal endothelial cells (LSEC) *in vitro*. However, it is not currently known how the SASP interacts with LSEC and which molecules/pathways are involved in the specific recruitment of $CD4^+$ T cells to eliminate OIS cells from the liver.

Method: We derived SASP from ER:Ras^{G12V} IMR90 cells (Ras) and control supernatants from growing IMR90 cells (Grow) and challenged LSEC for 24 h. Following this, we utilised RNAseq to explore gene regulation and gene set enrichment analysis was used to identify key pathways regulated. qPCR, immunocytochemical (ICC) staining, pharmacological inhibition and genetic manipulation of endothelial cells were all used to confirm findings from the RNAseq analysis. Next, we utilised flow-based adhesion assays with LSEC to study target molecules in the recruitment of $CD4^+$ T cells under physiological flow conditions *in vitro*.

Results: Using RNAseq, we identified inducible T cell co-stimulator ligand (*ICOSLG*) as a key gene upregulated in LSEC in response to Ras stimulation and confirmed this via qPCR. We next elucidated a novel role for ICOSL in the specific recruitment of $CD4^+$ T cell to LSEC. Using antibody blockade and siRNA knockdown of ICOSL expression in Ras-treated LSEC, we demonstrated significantly reduced transendothelial migration of $CD4^+$ T cells under physiological shear stress. In corroboration with these findings, we also found ICOSL-dependent trafficking of intercellular adhesion molecule (ICAM)-1 to the cell surface under Ras stimulation. siRNA inhibition of ICOSL reduced the number of ICAM-1 rich transcellular channels in LSEC, thus preventing $CD4^+$ T cell transmigration.

Conclusion: Here, we show that the SASP regulates endothelial activation and expression of co-stimulatory molecules, such as ICOSL, on primary human LSEC. We now demonstrate that levels of endothelial ICOSL regulates the function of conventional adhesion molecules, such as ICAM-1, in the specific recruitment of $CD4^+$ T cells. This could be a unique pathway by which the SASP regulates the immune microenvironment of the liver.

P05-15YI Activated platelets drive carcinogenesis and tumor growth in hepatocellular carcinoma

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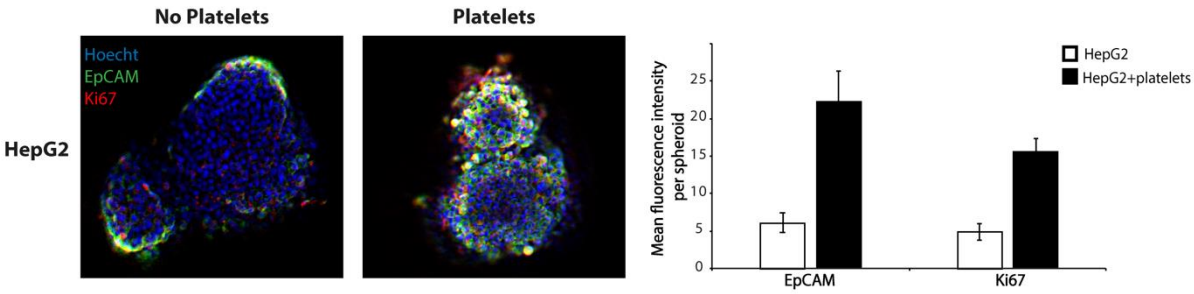
Background and aims: Hepatocellular carcinoma (HCC), an aggressive primary liver cancer, is one of the most common malignancies worldwide, with limited therapeutic options and an increasing mortality rate. The complex interplay between tumor cells and different factors of the HCC microenvironment have a crucial role in promoting disease progression. Platelets have emerged as potent drivers of hepatocarcinogenesis, as they have been reported to interact with key players of the tumor stroma, such as tumor cells, hepatic stellate cells (HSC) and macrophages. In this study, we aimed to determine the effect of platelets on HCC development, and whether inhibiting platelet activation can be therapeutically relevant for patients with liver cancer.
Method: Mice were injected weekly with diethylnitrosamine to induce HCC. From week 10, mice were treated with anti-platelet drug Clopidogrel or control. Samples were taken for histological, protein and RNA-analysis after 25 weeks. Tumour burden and collagen deposition were quantified on H&E and Sirius Red staining, respectively. Immunohistochemistry with CD41 and F4/80 antibodies was performed to quantify the number of platelets and macrophages in the liver, respectively. Fluorescently labelled HCC-cells (HepG2) and THP-1-differentiated macrophages were co-cultured with platelets and phagocytosis was observed. Decellularized human liver scaffolds were engrafted with macrophages, HepG2 cells and platelets, after which samples were taken for histological analysis. HepG2 cells and HSC (LX2) were grown as 3D spheroids in mono- and co-cultures, and exposed to platelets for 24 hours, after which immunocytochemistry was performed.
Results: Clopidogrel treatment significantly reduced the number of tumors and collagen deposition in mice with HCC. Immunohistochemistry showed a significant reduction of platelet and macrophage number in the liver of the diseased mice after treatment. Clopidogrel treatment increased the mRNA expression of several anti-tumoural markers (IL-1α, TNFα, iNOS and CD40). When co-cultured with tumor spheroids, platelets increased the protein expression of pro-tumoural markers ki67 and EpCam in HepG2 cells (figure), and protein expression of collagen and ki67 in HepG2/LX2 co-cultures. Performing a tumor phagocytosis assay and determining cell count on liver scaffolds showed that platelets inhibit macrophage phagocytosis of tumor cell.
Conclusion: This data shows that platelets play an important role in promoting HCC progression, by interacting with several players of the tumor microenvironment, including tumor cells, HSC and macrophages. Therefore, anti-platelet drugs may be clinically relevant for patients with HCC.

Figure:



P05-16 Vascular invasion, portal vein thrombosis and dermatological events predict and prognosis the evolution and survival of patients with advanced hepatocellular carcinoma treated with sorafenib

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Background and aims: Hepatocellular carcinoma (HCC) is characterized by a poor prognosis and survival, but some patients have a better response to sorafenib and higher overall survival (OS). The present study analyze the portal vein thrombosis (PVT) and vascular invasion (VI) as baseline prognostic factors, and evaluate dermatological events (DE) as a predictive factor of response to treatment in patients with HCC treated with sorafenib in the context of a multicenter phase II clinical trial.
Method: A phase II, multicenter, randomized, double-blind, placebo-controlled clinical trial was conducted in patients with advanced HCC candidates for treatment with sorafenib, with preserved liver function (Child-Pugh A or B7). They were randomized (1: 1) to receive sorafenib + placebo (control group) and sorafenib + pravastatin (experimental group). The maximum duration of treatment was 18 months (M). Variables were recorded every 8 weeks since the baseline visit. Survival data were analyzed using Kaplan-Meier method, compared by log-rank test and adjusted with a Cox regression model. The patients were divided into groups according to presence / absence of the studied factor.
Results: 31 patients were randomized. The clinical characteristics were the same in both arms. Mean age 61 years, 93% men, 90% Child A, 77% BCLC C, 42% with VI and 35% with PVT. Median OS was 12.4M. Presence of PVT was associated with OS (p = 0.026), being significantly lower in patients with PVT (6.3M, 95% CI 5.3-7.3) compared to patients without PVT (14.8M , 95% CI 11.6-18.0). Results showed a decrease in OS in patients with VI (6.3M, 95% CI 5.2-7.4) compared to patients who did not present it (14.8M, 95% CI 11.8-17.8); p = 0.041. 51.6% of the patients presented DE during the study, mainly hand-foot syndrome, rash and pruritus. The group of patients without DE showed a lower OS (6.9M, 95% CI 4.1-9.6) compared to the group of patients with DE (14.5M, 95% CI 11.6-17.3), p = 0.049. There were no differences between control and experimental groups for the studied factors.
Conclusion: The absence of portal vein thrombosis and vascular invasion are important baseline prognostic factors, significantly associated with higher survival. Dermatological adverse events is a predictive factor increasing OS of patients with advanced HCC in treatment with sorafenib, implying close monitoring and adequate dose adjustment to avoid interruption of sorafenib in a probably responding patient.

P05-17 Hepatotoxicity from immune-checkpoint inhibition in the treatment of hepatocellular carcinoma: Outcomes and tissue biomarker analysis

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Background and aims: In hepatocellular carcinoma (HCC) patients, factors leading to immune-checkpoint inhibitors (ICI)-related severe hepatotoxicity remain poorly understood. We sought to assess: (i) incidence and clinical predictors of immuno-related hepatitis during ICI, (ii) relationship between hepatitis grade and subsequent treatment outcomes, (iii) morpho-pathological factors linked to hepatitis.

Method: 58 patients with advanced/unresectable HCC and preserved liver function received anti-programmed cell death protein 1 (PD-1)/PD ligand 1 (PD-L1) antibodies. Hepatitis was graded according to National Cancer Institute's Common Terminology Criteria for Adverse Events. Tumor necrosis, intratumoral tertiary lymphoid structures (TILs), expression of CD34, Glutamine Synthase (GS), CD3 and CD79 were evaluated on 27 pretreatment tumor biopsies. GS immunoreactivity (any pattern) was used to discriminate an Immune class ("hot" HCC) from an Exclusion class ("cold" HCC).

Results: 20 patients received anti-PD-1/PD-L1 antibodies alone, and 38 in combination with anti-cytotoxic T-lymphocyte-associated protein 4 antibodies and/or tyrosine kinase inhibitors. After a median time of 0.9 months, 9 (15.5%) developed grade ≥3 hepatitis, which was not associated to any etiologic nor clinical parameter, except baseline ALT levels (p = 0.037). Steroids were administered in 3 patients and ICI were safely resumed in 6 out of 9 patients. No significant differences in time to treatment failure (TTF) were seen with grade ≥3 hepatitis vs lower grades (3.25 vs 3.91 months, respectively; p = 0.81). Immunoreactivity to GS, absence of TILs, and necrosis were more frequent in patients developing grade ≥3 hepatitis (p = NS; Table).

Conclusion: Upon resolution of hepatitis to grade ≤1, treatment with ICI can be safely reintroduced with no detrimental effect on TTF. Preliminary analyses suggest that some biomarkers could be linked with onset of high-grade hepatitis, though these findings need to be explored in larger series.

Table: HCC morpho-phenotypical features according to the severity of ICI-related hepatitis

Tumor features	Grade ≥3 Hepatitis (N = 7)	Grade 0-2 Hepatitis (N = 20)	p
GRADE ≥3	2 (28%)	7 (35%)	1
NECROSIS	3 (42%)	3 (15%)	0.290
TILs	0	3 (15%)	0.545
VETC	5 (71%)	13/19 (68%)	1
EXCLUSION CLASS	7 (100%)	16 (80%)	1
IMMUNE CLASS	0	4 (20%)	0.545
AFP >400ng/ml	2/5 (40%)	3/15 (20%)	0.545

P05-18YI Serum metabolic biomarkers for the differential diagnosis of distal cholangiocarcinoma and pancreas ductal adenocarcinoma

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Background and aims: The accurate diagnosis of adenocarcinomas located in the head of the pancreas -distal cholangiocarcinoma (dCCA) and pancreas ductal adenocarcinoma (PDAC)-, represents a clinical challenge since both types of tumors share symptoms and they cannot be distinguished by imaging techniques. At present there are no accurate serum biomarkers that allow early and differential diagnosis of these tumors. Omics technologies facilitate the profiling and analysis of disease-specific signatures and are powerful sources of candidates. The aim of this study was to determine serum metabolomics profiles in patients with dCCA or PDAC to identify novel biomarkers for the early and differential diagnosis.

Method: Chloroform/methanol and methanol extracts were obtained from the serum of patients with diagnosis of dCCA (n=34) or PDAC (n=38) confirmed by histopathology, attended in the University Hospitals of Donostia and Salamanca, and healthy individuals (n=25) divided in two cohorts. Ultra-high performance liquid chromatography coupled to mass spectrometry (UHPLC-MS) was used to determine amino acids and lipids.

Results: A total of 484 metabolites in serum samples were identified in both cohorts and included in the univariate and multivariate data analyses. Compared to controls, serum samples of patients with dCCA and PDAC had higher levels of several metabolites, mainly triglycerides, diglycerides and bile acids in dCCA and triglycerides, diglycerides and diacylphosphatidylethanolamines in PDAC. Fewer changes were found between the circulating metabolomes of dCCA and PDAC, although several species of phosphatidylethanolamines (PE), lysophosphatidylethanolamines and triglycerides were more elevated in PDAC than in dCCA.

Among other metabolites, glutamic acid and aspartic acid distinguished tumors from controls with an AUC> 0.91 and 0.94, respectively. To determine a predictive model for the discrimination between patients with dCCA and PDAC based on circulating metabolites both cohorts were merged and divided into three cohorts: training (70%) and validation (20%) for cross-validation and parameter optimization and test (10%) for blind validation. A logistic regression model with six metabolites [PC(17:0/0:0), PI(18:0/20:3), 12-HETE, PE(0:0/16:0), hydrocortisone and phenylalanine] was found, with an AUC of 0.92 in training, 0.84 in validation and 0.85 in test cohorts.

Conclusion: Specific panels of serum metabolites can be useful to distinguish dCCA from PDAC. Validation of the clinical usefulness of these biomarkers in further prospective studies is granted.

P05-19YI SCCA-IgM predicts prognosis differently according to gender in hepatocellular carcinoma patients treated with transarterial chemoembolization

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Background and aims: The determination of Squamous Cell Carcinoma Antigen (SCCA)-IgM complex in serum is able to predict hepatocellular carcinoma patients' prognosis. From the biologic point of view, gender has an impact on serpins molecular activity, but no studies evaluated the predictive capacity of this circulating biomarker according to sex. Aim of our study was to investigate gender-related differences in SCCA-IgM determination, in particular regarding its prognostic role, in hepatocellular carcinoma (HCC) patients treated with transarterial chemoembolization (TACE).
Method: A total of 208 consecutive HCC patients treated with TACE were retrospectively evaluated. A blood sample was collected for every patient immediately before TACE and in a subgroup of 149 a second sample was obtained 4 weeks after the treatment, at the time of the radiological control. SCCA-IgM was measured in serum with an ELISA method. The value of 130 AU/mL, previously validated, was chosen as prognostic cut-off. The difference between pre- and post-TACE values (ΔSCCA-IgM) was considered relevant in case of a variation of at least 25% with respect to the basal levels. Association with clinical and tumoral characteristics, response to treatment and survival was evaluated.
Results: The male and female subgroups differed in sample size (80% males and 20% females), age, etiology, MELD, MELD-Na, number of nodules, presence of metastases and AFP levels. SCCA-IgM levels were not different according to gender. Higher SCCA-IgM levels were detected in males with advanced ITALICA prognostic score (> 3) and in females with earlier stage tumors (≤ 3). SCCA-IgM levels, either before and after TACE, and ΔSCCA-IgM were not associated with radiological response (mRECIST). At the established cut-off (130 AU/mL), SCCA-IgM was not efficient in predicting the prognosis in the overall population. However, when males and females were considered separately, an opposite predictive capacity was evident: a longer overall survival was observed in males with SCCA-IgM levels below the cut-off (median OS of 35.7 vs. 20.8 months; p = 0.007); in contrast, females with SCCA-IgM levels < 130 AU/mL showed worse prognosis (15.7 months vs. 36.4 months; p = 0.01) (see Figure).
Conclusion: SCCA-IgM, a useful biomarker in HCC, predicts survival differently according to gender. More studies are needed to confirm our data and identify the mechanisms underlying this different, gender-specific, behavior.

Figure:

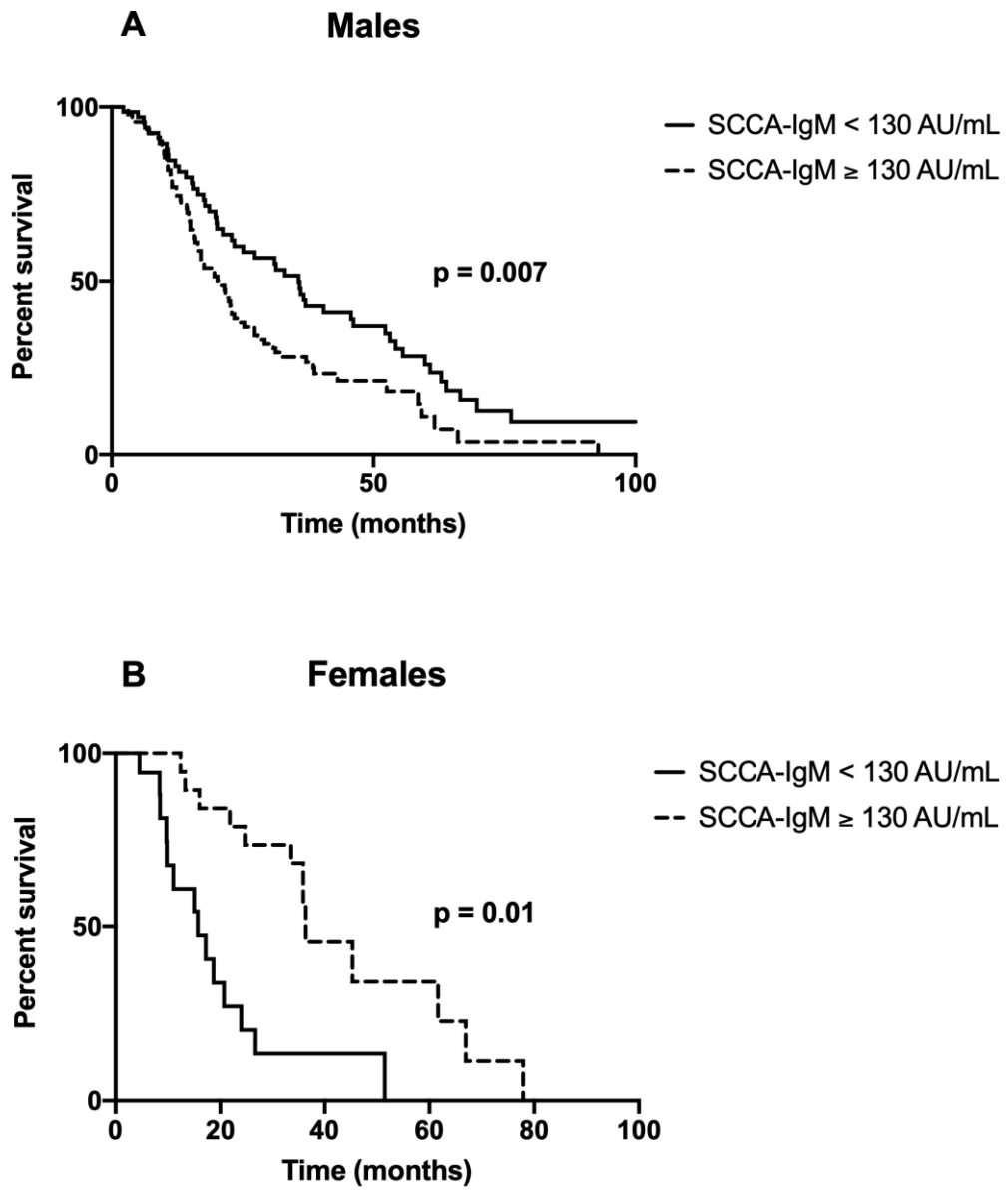

Figure. Kaplan-Meier curves showing overall survival according to SCCA-IgM levels in males (A) and females (B)

P05-20YI Differential role of TGF-beta1&2 on proliferative and invasive properties in primary liver cancer

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Background and aims: Transforming Growth Factor Beta (TGF-β) belongs to a superfamily of cytokines that induces pleiotropic effects on different processes and cell types in the liver. While TGF-β signaling exerts tumor suppressive functions at pre-neoplastic and early tumor stages, cytostatic effects of TGF-β are often lost in progressed stages due to (epi-) genetic disruption of several members of the signaling pathway. This progressed stage is characterized by activation of a "late TGF-β signature" which promotes the phenotypic switch from tumor suppressor to promoter of cancer.
Method: Primary patient-derived and established cell lines were exposed to TGF-β1 and TGF-β2 (1ng/ml) for 72 hr. The effect of TGF-β on tumor-initiating potential was determined by colony and sphere formation assays. Invasive and migratory properties were determined using the wound healing assays. Transcriptomic and proteomic analysis was performed to explore differential gene/protein expression between treatments.
Results: Treatment with TGF-β1 and TGF-β2 led to a significant reduction in colony and spheroid forming ability in all investigated cell lines. Interestingly, a significant downregulation of epithelial marker E-cadherin and concomitant upregulation of mesenchymal markers such as Vimentin and SNAIL was exclusively observed after TGF-β1 treatment. Similarly, a significant increase in migratory and invasive properties of HCC and CCA cell lines was induced by TGF-β1 whereas TGF-β2 treated cells showed no effect. In addition, transcriptome and proteomic profiling confirmed activation of gene sets and pathways involved in Cell Cycle:G1/S Checkpoint in response to both treatments(TGF-β1&β2) whereas enrichment in signaling pathways known to be involved in pro-metastatic properties resembling MMPs, Hippo pathway etc were predominantly associated with the TGF-β1 response.
Conclusion: We here confirm the cytostatic effect of TGF- β1 and TGF- β2 in primary liver cancer. Further, TFG-β1 is an important regulator of EMT in progressed PLCs and induces migratory and invasive properties.

P05-21YI Critical modifications of ganglioside patterns in human cholangiocarcinoma

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Background and aims: Cancer stem cell (CSC) represents a critical therapeutic target in neoplastic diseases. Gangliosides (GS) are a family of sialic acid–containing glycosphingolipids, which have been associated with the malignant phenotype of several cancers (i.e. breast, melanoma, glioblastoma, ovary). Recent evidence indicates the possible involvement GS in tumor stem cell biology, but no data regarding GS composition in human cholangiocarcinoma (CCA) are available. The aim of this study was to provide a GS profiling of the stem-like subset and of their parental cells in human CCA.

Method: Stem-like subset was enriched by sphere culture (SPH) using well-established human intrahepatic CCA cells (HUCCT1, CCLP1). CCA GS patterns were analyzed by chromatographic procedures. Assessment of GS turnover and identification of GS molecular species were evaluated using 3Hsphingosine. The role of GS in the modulation of stem features was investigated using D-threo-1phenyl-2-palmitoylamino-3-N-morpholine-1-propanol (PPMP), a GM3 synthase inhibitor. FACS-sorted GD2+ SPH cells were examined for stem-like gene expression compared to GD2- SPH.

Results: In both CCA lines, the amount of total GS was markedly different comparing parental cells grown in monolayer conditions (MON) and their stem-like subsets (SPH). CCA-SPH showed enrichment in the major GS class (GM3), reduction of GM2 and the presence of GD2. This was corroborated by high expression levels of GM3 synthase as well as of GD3 and GM2/GD2 synthases in CCA-SPH. Enzymes involved in GS biosynthesis were strongly expressed in CCA-SPH compared to MON. Notably, sphere-forming ability and expression of CSC-related genes were reduced in cells treated with by PPMP. Likewise, GD2+ SPH cells were enriched with CSC-markers (CD133, EpCAM, CD44) and expressed at higher levels several genes involved in pluripotency, self-renewal and epithelial mesenchymal transition compare to GD2- SPH. Notably, expression of GM2/GD2 synthases was significantly increased in tumor samples compared to paired non-tumoral tissue of CCA patients (n=104) and significantly correlated with the presence of satellite nodules, lymph node invasion and recurrence.

Conclusion: We show for the first time that the CCA stem-like properties may be associated with modification in the GS synthetic pathway

P05-22YI Applicability of "Six and Twelve" model in patients with hepatocellular carcinoma treated with DEB-TACE

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Background and aims: The effectiveness of transarterial chemoembolization (TACE) in hepatocellular carcinoma (HCC) depends on the selection of suitable patients. The “Six-and-Twelve” model [Wang Q et al, J Hepatol. 2019; 70(5): 893-903] has been developed and validated in an Asian cohort of ideal TACE candidates, and it distinguishes three groups with different overall survival (group 1, <6 points; group 2, 6-12; group 3, >12). This stratification based on the sum of number and size of tumors may impact in clinical practice and trials design. The score has been recently validated in a multicentric French cohort of patients treated with conventional TACE (n=127) [Bourlière M et al. J Hepatol. 2019;71(5):1051-1052]. The aim of this study is to evaluate de usefulness of the “Six-and-Twelve” model in a cohort of HCC patients treated with DEB-TACE.

Method: Observational retrospective study with consecutive HCC patients treated with DEB-TACE from October/2008 to October/2017; end of follow-up October 24th 2019. Exclusion criteria were Child-Pugh not available or ≥ 8, patients awaiting liver transplantation.

Results: 225 patients made up the study cohort: 187 men; 107 alcohol etiology, 70 HCV; Child A-5 n=165, A-6 n=43, B-7 n=17; BCLC-0 n=10, BCLC-A n=102, BCLC-B n=113. Median diameter of main nodule: 3.5 cm (IQR 2.5-4.8); median number of nodules 2 (IQR 1-3). Median overall survival (OS) was 27 months (95% CI 24.042-29.958), without differences in OS (25 months, 95% IC 20.734 – 29.266 vs 27 months, IC 95% 23.458 – 30.542, p= 0.586, respectively) between those with / without prior history of cirrhosis decompensation 34% (n=77) and 66% (n=148), respectively. OS was different between BCLC-0-A vs B: 32 vs 24 months, p= 0.004, and in Child-Pugh A5 compared to A6-B7: 30 vs 27 months, p= 0.005. The median value of the “Six-and-Twelve” score was 6 (P_{25}-P_{75} 4.5-7.4). OS was different by stratifying through “Six-and-Twelve” model: group 1, n=123, 32 months (95% CI 25.893-36.107) vs group 2, n=101, 24 months (95% CI 19.576-28.424) vs group 3, n=1, 27 months (p=0.048).

Conclusion: The “Six-and-Twelve” model is a useful prognostic tool in patients with cirrhosis and HCC treated with DEB-TACE. As well as in the French cohort only few patients are included in the third group (score >12), therefore, the applicability of this model in our region is limited.

P06-01YI Circulating microRNA-21 and microRNA-122: prognosis prediction and correlation with HIF-1alpha in hepatocellular carcinoma patients treated with transarterial chemoembolization

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Background and aims: MiR-21 and miR-122 have been identified as promising circulating biomarkers in hepatocellular carcinoma (HCC). We aimed to evaluate the prognostic role of their determination in HCC patients treated with transarterial chemoembolization (TACE), a treatment that induces liver ischemia, and the link, if present, with an angiogenesis biomarker (HIF-1alpha).

Method: Whole blood levels of miR-21 and miR-122 were evaluated in 40 HCC patients, 18 cirrhotics and 10 healthy volunteers, with a second determination in HCC 4 weeks after treatment (at the time of the imaging control). The miRNA level before TACE and the miRNA ratio (miRNA after/before TACE) were evaluated as potential progression-free survival (PFS) predictors. The correlation between miRNAs and HIF-1alpha was established using the Spearman's rank correlation coefficient. MiRNA levels were evaluated with qRT-PCR and expressed as $2^{-\Delta\Delta Ct}$; an ELISA method was used to measure HIF-1alpha levels.

Results: Both miR-21 and miR-122 were detectable in the blood of HCC patients at significantly higher levels as compared to healthy controls, with no significant differences with cirrhotics. A trend towards a decline in miR-21 after TACE ($p = 0.056$) was observed; miR-122 levels, despite being higher after TACE, were not significantly different. MiR-122 was higher in HCC patients with underlying viral liver disease ($p = 0.03$). High miR-21 levels before TACE (> median value) predicted favorable radiological response (chi-squared = 4.8; $p = 0.03$). MiR-21 ratio and miR-122 before TACE proved to be prognostic predictors: patients with levels of miR-21 ratio and miR-122 below the respective cut-off had a longer PFS ($p = 0.0001$ and $p = 0.009$, respectively) (see Figure). MiR-21 ratio, miR-122 and radiological response (mRECIST), were independent predictors of PFS at the Cox multivariate analysis. Moreover, miR-21 ratio and miR-122 were able to sub-stratify patients with complete or partial response in two groups, at favorable or poor PFS. MiR-21, but not miR-122, positively correlated with HIF-1alpha both before ($r = 0.34$, $p = 0.049$) and after TACE ($r = 0.42$, $p = 0.01$).

Conclusion: MiR-21 and miR-122 are independent predictors of PFS in TACE-treated HCC patients and are able to identify, in patients with favorable radiological response, those with an early tumor progression after the treatment. The finding of a link between circulating miR-21 and HIF-1alpha in HCC indicate a potential role of miR-21 in angiogenesis.

Figure:

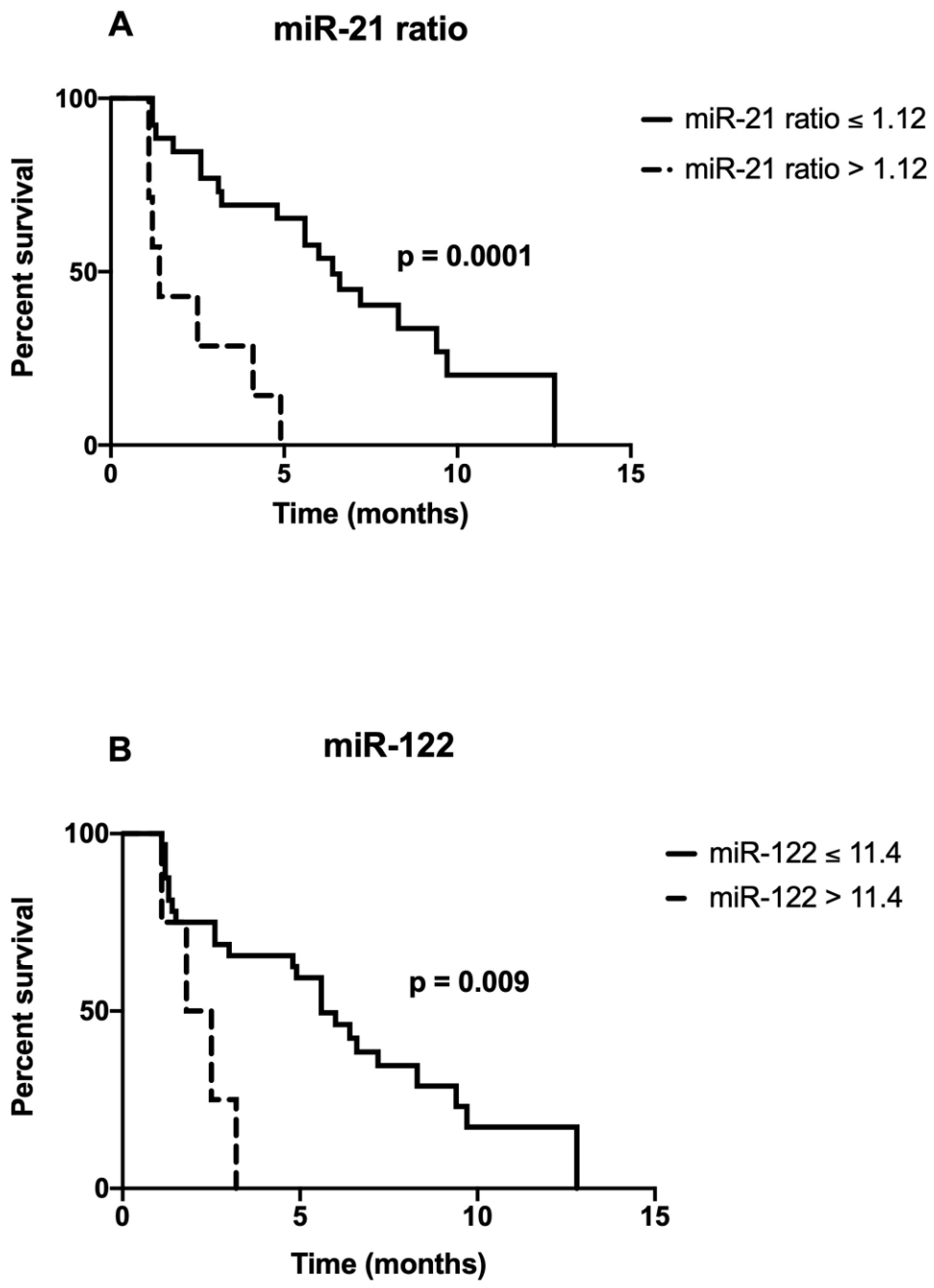

Figure. Kaplan-Meier curves showing PFS according to the levels of miR-21 ratio (A) and miR-122 (B)

P06-02YI Metabolic rewiring in tumoral primary cholangiocytes: perspective for target therapy

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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 tumor 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 both tumoral and non-tumoral primary BECs by profiling both the extra- and endo-metabolome.
Method: Primary non-tumoral BECS (NT-BECs) and tumoral iCCA BECs (iCCA-BECs) were isolated from 15 patients surgically resected at the Division of Hepatobiliary and General Surgery, Humanitas Clinical and Research Center. Both tumoral and non-affected BECs from the same donor were cultured until reaching 80% of confluence. Cells and their conditioned medium were analyzed by using mass spectrometry-based untargeted and targeted metabolomics approaches to explore the main metabolic processes. Moreover, primary iCCA BECs and Hucc-T1, human iCCA immortalized cell line, were seeded in 96-well plates to perform proliferation assay at different time point with different culture medium to detail the involvement of nutrients in iCCA-BEC proliferation.
Results: We observed that iCCA-BECs were characterized by higher mitochondrial activity compared to NT-BECs in all samples, in which glutamine and pyruvate act as metabolic sources to fuel central metabolism respectively. Importantly, iCCA-BECs exposed to different nutrient environments were able to reprogram nutrient uptake and utilization to boost central cellular metabolism. Furthermore, the proliferation assay showed that iCCA-BECs, when cultured in a different metabolic medium composition, were able to exploit the different metabolic sources to sustain cancer cell growth.
Conclusion: This observation raises the prospect that interfering with mitochondrial activity of iCCA cancer cells could make them more susceptible to cytotoxic drugs, opening new possibility to improve the outcomes of the iCCA patients.

P06-03YI Circulating mir-4454 as a predictor of therapy response and disease-free survival in hepatocellular carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) represents the fourth leading cause of cancer-related death worldwide due to the late diagnosis and its poor prognosis rate. Non-invasive biomarker to predict outcomes for each therapies might be a feasible strategy to support the idea of individual treatment in order to improve the prognosis of HCC patients. In the present study, we conducted a longitudinal study to address the potential of miR-4454 as prognostic biomarker in HCC patients.

Method: A total of 105 patients with HCC were enrolled and treated according to the EASL/AASLD practice guidelines. Serum samples were collected before therapy (T0), one month (T1), and six months (T6) after therapy. We conducted a discovery phase from twenty serum samples using miRNA 3.0 gene array (Affymetrix) microarray profiling analysis. MiRNAs associated to therapy response (TR) and disease-free survival (DFS) were selected for subsequent validation by using qRT-PCR by usingmiR-1280 as internal normalizer. The receiver operating characteristic (ROC) curves were plotted to estimate the prognostic value of the miRNA.

Results: At T0, the expression of miR-4454 was 1.88 fold times higher in complete responder (CR) compared to partial and non-responder (PR) (p = 0.05). Considering patients treated with curative therapies (n = 41), miR-4454 was significantly higher in CR at all considered times (p= 0.0009). The predictive value of miR-4454 to identify CR before therapy showed an AUC of 0.74 (0.49 - 0.88 95% CI, 79% sensitivity, 63% specificity). Patients with high miR-4454 showed a disease free survival (DFS) longer than 6 months (p = 0.05). Mir-4454 was able to distinguish patients with Longer DFS before curative treatment (AUC= 0.73, 0.50 - 0.86 95% CI, 67% sensitivity, 64% specificity). Kaplan-Meier survival analysis demonstrated that high expression of miR-4454 was significantly associated with longer overall survival (HR=2.63, 0.15-0.9395% CI, p=0.016).

Conclusion: Serum miR-4454 has a potential value as a novel biomarker to predict therapy response and disease-free survival before the curative treatments in patients with HCC.

P06-04YI Characterization of novel oncogenic and tumor suppressor circRNAs in hepatocellular carcinoma

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Background and aims: Circular RNAs (circRNAs) represent a new class of covalently closed single-stranded RNAs. The role of some circRNAs in hepatocellular carcinoma has already been studied, however, clinical significance and role of the majority of differentially expressed circRNAs remains to be evaluated. Since they show tissue specific expression and are more stable than linear transcripts, they represent a group of promising biomarkers. Aim of the research was to characterize oncogenic or tumor suppressor potential of new candidate circRNAs and establish methods for the isolation of extracellular vesicles for further assessment of their diagnostic potential.
Method: To identify new potential oncogenic or tumor suppressor circRNAs in hepatocellular carcinoma we analyzed two datasets of circRNA microarrays from GEO database, in which cancerous and distant paracancerous tissue samples were analyzed. Oncogenic and tumor suppressor potential of candidate circRNAs was evaluated in model cell lines Huh-7 and HepG2 by investigating the proliferation, migration and invasion of transfected cells. Extracellular vesicles were isolated from diluted plasma samples or culture medium by ultracentrifugation and characterized by nanoparticle tracking analysis, electron microscopy and western blotting.
Results: We have identified 32 upregulated and 6 downregulated circRNAs in both datasets. The predicted role of some circRNAs in oncogenesis was confirmed in transfected cells, where they affected the proliferation, migration and/or invasion. We have also successfully established a method for isolation of extracellular vesicles from model cell lines and human plasma samples and detection of circRNAs in extracellular vesicles.
Conclusion: By comparing the differential expression of circRNAs in two microarray datasets we have found new potential tumor suppressor and oncogenic circRNAs, of which some were confirmed to have a role in cell-based assays. We have also successfully isolated and characterized extracellular vesicles from cell culture medium and plasma samples. In our future work we intend to investigate the mechanism of action of candidate circRNAs and evaluate their diagnostic potential in extracellular vesicles of hepatocellular carcinoma patient`s plasma samples.

P06-05YI Prognostic role of a new index (PECS index) in advanced biliary tract cancer patients treated with first line chemotherapy: Training and Validation cohorts

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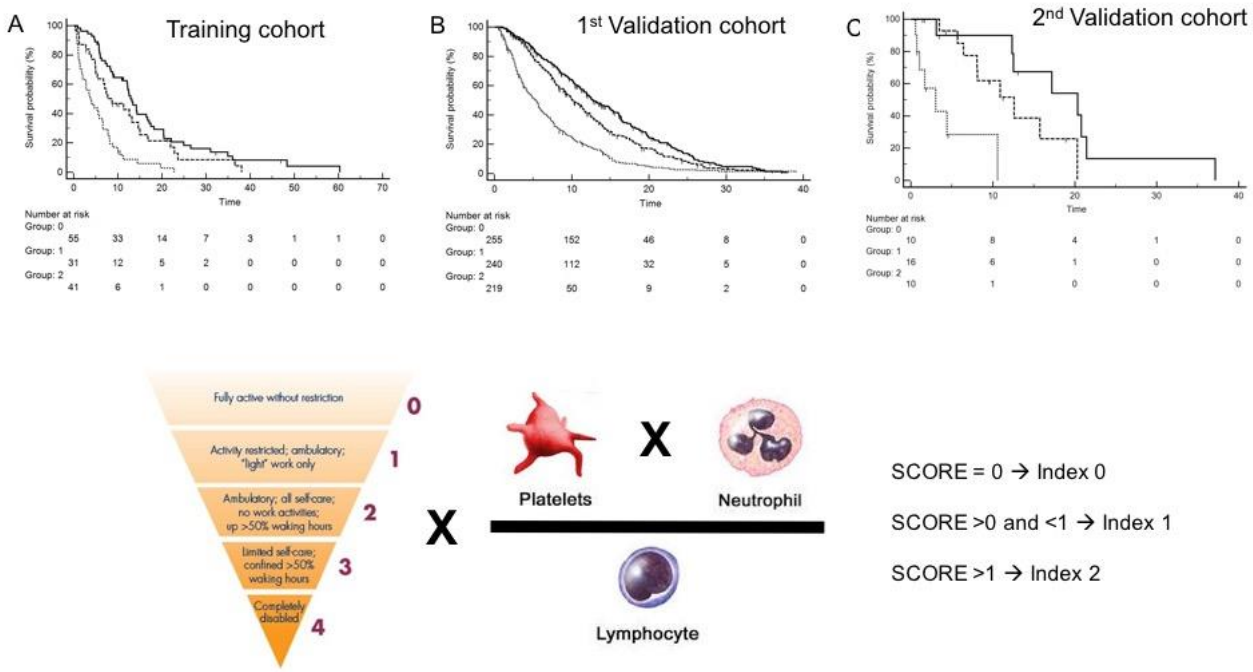
Background and aims: Biliary tract cancer (BTC) is a relatively rare malignancy for which few treatment options are available. In this context, prognosis estimation at initial presentation, thus facilitating treatment optimization, is essential. However, still no clinical validated prognostic indexes are available in routine clinical practice. The aim of the present study is therefore to evaluate a new index influenced by PS ECOG and immune system as prognostic factor in unresectable locally advanced or metastatic BTC patients treated with first-line chemotherapy. This new factor is named PECS (PsECogSii) index.
Method: This retrospective study was conducted on a training cohort of 127 BTC unresectable locally advanced or metastatic patients treated with first-line chemotherapy at Modena Cancer Center. According to the same inclusion criteria, a first validation cohort of 714 patients was consecutively recruited by ten Italian Cancer Centers and a second validation cohort of 36 patients by University of Cagliari. For every patient biological/clinical parameters were collected the day before the start of the treatment. The PECS index was calculated as PS ECOG x SII (platelets x neutrophil/lymphocyte) count. Event-time distributions were estimated using the Kaplan-Meier method and survival curves were compared using the log-rank test.
Results: In the training cohort, the median overall survival (OS) was 13.2 months (95%CI 11.2 - 60.3), 8.7 months (95%CI 5.0 – 38.0) and 3.8 months (95%CI 1.9 – 22.7) for patients with PECS-0, PECS-1 and PECS-2, respectively (PECS-0: HR=1; PECS-1: HR 1.41; PECS-2: HR 3.23) (p<0.0001). In the first validation cohort, the median OS was 12.8 months (95%CI 11.5 – 15.0), 10.1 months (95%CI 9.0 – 37.2) and 5.3 months (95%CI 4.2 – 39.0) for patients with PECS-0, PECS-1 and PECS-2, respectively (PECS-0: HR=1; PECS-1: HR 1.29; PECS-2: HR 2.40) (p<0.0001). In the second validation cohort, the median OS was 20.3 months (95%CI 3.1 – 37.1), 12.6 months (95%CI 6.4 – 20.3) and 3.0 months (95%CI 0.6 – 10.6) for patients with PECS-0, PECS-1 and PECS-2, respectively (PECS-0: HR=1; PECS-1: HR 1.70; PECS-2: HR 6.83) (p<0.0001). Multivariate analysis in all cohorts confirmed the PECS index as an independent prognostic factor for OS.
Conclusion: The low cost, easy assessment and reproducibility make PECS index a promising tool to assess the prognosis of BTC patients in future clinical practice.

Figure:


A
Training cohort
B
1st Validation cohort
C
2nd Validation cohort
Survival probability (%)
Time
Number at risk
Group: 0
Group: 1
Group: 2
Fully active without restriction
0
Activity restricted; ambulatory; "light" work only
1
Ambulatory; all self-care; no work activities; up >50% waking hours
2
Limited self-care; confined >50% waking hours
3
Completely disabled
4
X
Platelets
X
Neutrophil
Lymphocyte
SCORE = 0 → Index 0
SCORE >0 and <1 → Index 1
SCORE >1 → Index 2

P06-06 Hepatic lesion involving the inferior vena cava: surgical management

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Background and aims: Liver tumors involving the inferior vena cava (LTIVC) often required combined liver and caval (IVC) resection with direct suture (DS) or graft reconstruction, with or without total vascular exclusion (TVE).
Method: 1495 consecutive patients underwent liver resection from December 2007-June 2019; 187 LTIVC were analyzed. Mean age was 61 years; 109 men, 78 women. Histology was: hepatocellular-carcinoma (37), colorectal-liver-metastases (111), mass-forming-cholangiocellular-carcinoma (22) and others (17). Tumor mean size was 48 mm.
Tumor detachment (LD) was performed in 152 patients, partial resection and DS IVC reconstruction (PR) in 35.
We defined 3 groups according to IVC circumferential involvement: <60° (A), 60-120° (B), >180° (C).
Results: All patients underwent intermittent warm ischemia. Sixteen had TVE: 6, 7 and 3 in A, B, C respectively. PR was performed in 14/112 (12.5%), 13/55 (23.6%), 8/20 (40%) in A, B, C respectively. Ten patients had ICV thrombi. No IVC replacement was performed.
Mortality rate was 3.2%, 5 in LD, 1 PR.
Major morbidity occurs in 18 patients (9.6%): 11 in LD, 7 in PR; minor morbidity in 63 patients (33.7%): 54 (35.5%) in LD, 9 (25.7%) in PR.
Median overall survival was 20 months: 21 in LD, 18 in PR.
Local recurrence was 23.5%: 31 (20.4%) in LD, 13 (37.1%) in PR.
Conclusion:
LD from IVC with or without PR is safe and effective: the first option even in case of lesions with an extensive involvement of IVC. IVC replacement should be an exceptional procedure.

P06-07YI Multicenter validation of a scoring system to predict survival in biliary tract cancers receiving second-line chemotherapy

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Background and aims: The role of second-line chemotherapy in BTCs has only recently been established in the phase III randomized ABC-06 trial. However, the optimal selection of patients most likely to benefit from second-line treatment remains challenging. We aimed at externally validating our previously-developed prognostic model which includes eight variables, i.e. progression-free survival to first-line, ECOG PS, peritoneal carcinomatosis, LDH, albumin, sodium, lymphocyte count, gamma-GT (Salati M. et al. ASCO GI 2019).
Method: Patients with BTCs treated with second-line chemotherapy between 2006 and 2018 were retrospectively identified from the following Italian Centre: Milano, Napoli, Cefalu', Trento and Cremona. Medical records were retrieved and patients with all the eight variables available before starting treatment made up the validation cohort (VC). The primary endpoint was overall survival (OS) and performance measures were applied to assess applicability of the model.
Results: The VC included a total of 120 patients. Median age was 65 years old, 61 were male (51%). Globally, 64 (54%) patients had intrahepatic cholangiocarcinoma, 35 (29%) gallbladder carcinoma and 21 (23%) extrahepatic cholangiocarcinoma. A total of 78 (65%) patients received multiagent chemotherapy, among which mFOLFIRI (35%) was the most commonly adopted regimen, followed by mFOLFOX (29%). The median OS was 7.5 months (95%CI 5.9-8.6 months) and 1-year OS was 17.5% (95%CI 10-24). Regarding prognostic variables of interest: 80 (67%) patients had ECOG PS >0, 30 (25%) had peritoneum involvement, 56 (47%) LDH >430 U/L, 66 (55%) had albumin <3.5 g/dL, 30 (25%) had gamma-GT >100 UI/L, 50 (42%) had progression-free survival to first-line < 6 months, 42 (35%) Na+ <140 mEq/L, 71 (59%) absolute lymphocyte count < 1000 /uL. In the VC, 25 (20%), 47 (40%) and 48 (40%) patients were assigned to the low-, intermediate- and high-risk group, according to the presence of 0-2, 3-4 and 5-8 risk factors, respectively. The score was able to identify prognostic classes with different median OS: 12.8 months (7.4-11.0), 6.4 months (5.2-8.3) and 2.5 months (1.8-2.7), in the low-, intermediate- and high-risk groups, respectively ($p<0.001$). The performance of the prognostic model in the VC was adequate, in terms of both discrimination (c-Harrell index 0.712; D-Royston 1.467) and prediction accuracy (calibration slope 0.65, $p<0.001$).
Conclusion: We externally validated our prognostic model in an independent multicentre cohort of BTCs receiving second-line chemotherapy. This could represent a valuable prediction tool to guide treatment decision in daily practice and risk-stratification clinical trials. Prospective validation in larger studies is warranted to strengthen our findings.

P06-08YI The "Six and Twelve" score in a prospective cohort of patients with hepatocellular carcinoma treated with trans-arterial chemoembolization following a fixed schedule

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Background and aims: Trans-arterial chemoembolization (TACE) is the standard of care treatment for patients with hepatocellular carcinoma (HCC) at BCLC-B stage or BCLC-0/A without other options while some BCLC-B patients receive systemic therapy as first-line treatment according to the stage migration concept. Several tools to better define the prognosis of TACE candidates have been proposed. The "Six-and-twelve" score stratifies TACE candidates into 3 strata (G1 ≤ 6; G2 >6 y ≤12; G3>12) according to the sum of the biggest target lesion size and the number of lesions. It stratifies overall survival (OS) with a reported C-index of 0.67 in Asian patients (ES ASI).
To assess the performance of the "Six-and-twelve" score to predict the OS in a prospective Western cohort of patients treated with fixed-schedule TACE at 0,2 and 6 months and thereafter every 6 months until untreatable progression.

Method: All TACE candidates at Hospital Clinic of Barcelona between 01/2014 and 03/2017 were prospectively included and the OS was analyzed according to the "Six-and-twelve" score in the whole cohort and in each BCLC stage.

Results: of the 105 included patients (HCV 51.89%, Child-Pugh-A 92.45% and BCLC-A/B 46/59), 90 received at least one treatment session. The median follow-up of the whole cohort was 24.4 months and the median OS (mOS) was 35.5 moths (IC95% 28.7– 42.6).
The "Six-and-twelve" score was G1 in 60 patients and G2 in 30 but no patient had G3. The mOS of G1 patients was 42.6 months (IC95% 29.8 – 49.0) and of G2 de 29.0 (IC95% 21.1 – 36.2) with a *p*-value = 0,037 and a *C-index* of 0.54 (IC95% 0.46 – 0.62). Thirty-eight G1 patients were BCLC-A and 32 BCLC-B, while only 5 of the 46 BCLC-A patients were G2. When the "Six-and-twelve" score was applied in each BCLC stage, the mOS was not different [(BCLC-A (p=0.39), HR=2.07 (IC95% 0.66 – 6.44); BCLC-B patients (p=0.82), HR=0.85 (IC95% 0.42 – 1.7)]. Specifically, the mOS of G1 and G2 were 44.0 months (IC 95% 36.3 - NE) and 36.2 (17.8 - NE) in BCLC-A patients and 27.6 (IC 95% 22.3 – 49.0) and 28.7 (IC 95% 18.4 – 35.5) in BCLC-B, respectively.

Conclusion: This study confirms the ability of the score to predict OS according but with a very low performance (*C-index* = 0.54). The score is not able to stratify TACE candidates in each BCLC stage. Thus, its clinical-practice usefulness remains limited although it could be considered as a potential tool to stratify TACE candidate in clinical trials.

P06-09YI Role of transient elastography in early detection of hepatocellular carcinoma in cirrhotic patients

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Background and aims: Hepatocellular carcinoma is common primary malignancy of the liver and one of the most frequent causes of death in patients with liver cirrhosis . Nowadays , liver stiffness measured noninvasively by transient elastography has been reported to be well correlated with histologically assessed liver fibrosis stage. The degree of liver fibrosis is the strongest indicator of risk for HCC development, that's why liver stiffness measured by transient elastography is helpful in demarcating patients at a high risk for HCC, who need frequent check-up by imaging examinations. Aim of the work was to study the role of ultrasound elastography (FibroScan) in early detection of hepatocellular carcinoma in cirrhotic patients as well as verifying whether ultrasound elastography (FibroScan), could be used as a tool for identifying cirrhotic patients who are at high risk of developing HCC.
Method: This study included 100 patients; 50 with HCC and 50 cirrhotics without evidence of HCC patients. For all groups, clinical data and image findings were studied. Tumour characteristics were assessed including size, number and site. Tumor staging was done using Okuda, CLIP, VISUM and Tokyo staging systems. Transient elastography was done for all patients and the results were expressed in kilopascal.
Results: Liver stiffness was significantly higher in HCC patients compared to cirrhotic patients. The sensitivity and specificity in diagnosis of HCC were 72% and 84% respectively at cutoff of 30.4 kpa with 91.1% accuracy. Fibroscan has a positive signifcant correlation with tumour size *(p=<0.001),* Child – Pugh *(p=<0.001)*, Okuda classification *(p=<0.001)*, CLIP staging *(p=<0.001)* and Tokyo classification *(p=<0.001)* among HCC patients. It was found that likelihood of HCC risk was correlated with increase of liver stiffness. At liver stiffness of 25-30 kpa the probability of HCC is 91% so, these patients should undergo close follow up. Patients with stiffness ≥30 kpa had HCC.
Conclusion: fibroscan could be used for early detection of HCC in cirrhotic patients and determining the patients who are at high risk for developing HCC.
Figure:

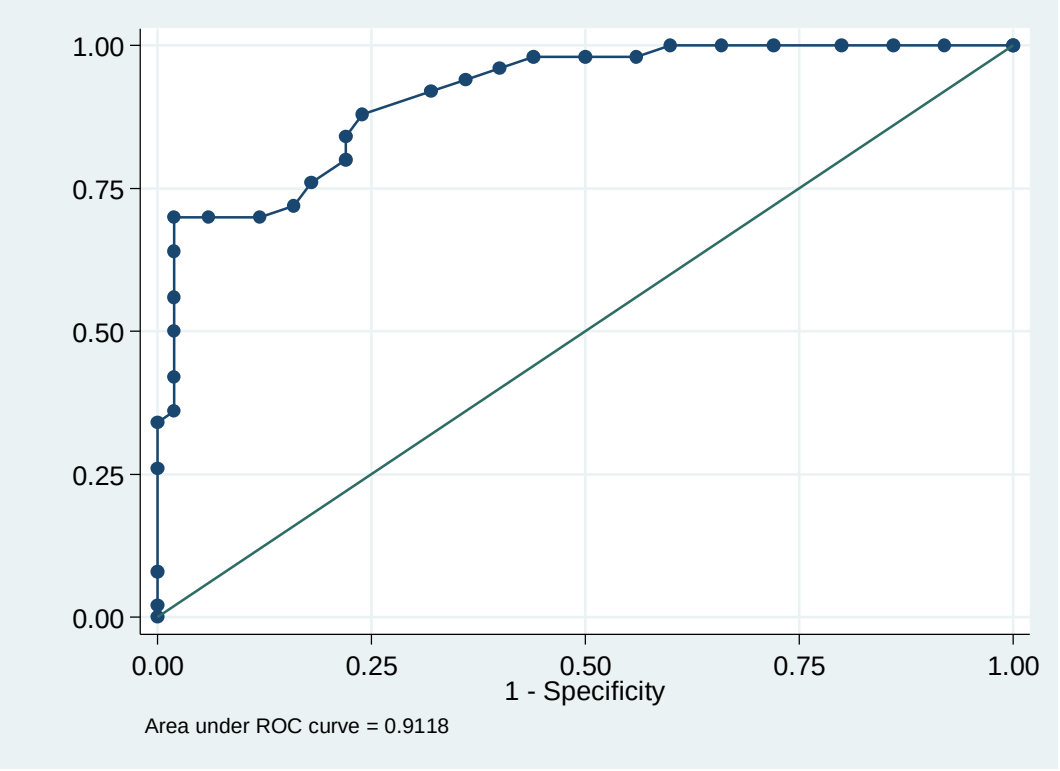

Figure (1) ROC curve analysis of Stiffness in prediction of hepatocellular carcinoma.

Stratum Specific Likelihood Ratio (SSLR) for HCC risk by measurement of liver stiffness:-

Strata	Non HCC (No.=50)		HCC (No.=50)		SSLR
	No.	%	No.	%	
<25	11	22.0	8	16.0	0.7272
25-30	12	24.0	11	22.0	0.9167
31-35	7	14.0	7	14.0	1.00
36-40	14	28.0	16	32.0	1.1428
>40	6	12.0	8	16.0	1.3333

P06-10 Genetic variants do not predict the development of hepatocellular carcinoma in cross-sectional and longitudinal studies including caucasian compensated HBV cirrhotics treated with NUC for 10 years

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Background and aims: Signal transducers and activators of transcription (STAT4), Epidermal Growth Factor 1 (EGF1), Tolloid like 1 gene (TLL1), Myeloid-epithelial-reproductive tyrosine kinase (MERTK) and for domain II (MERTK2), Patatin-like phospholipase-3 gene (PNPLA3) and Membrane Bound O-Acyltransferase Domain Containing 7 (MBOAT7) genetic variants have been associated with the development of hepatocellular carcinoma (HCC) in Asian and Caucasian HBV patients. Here we assess if these variants predict the HCC onset also in HBV cirrhotics long-term treated by NUCs.

Method: TDF or ETV-treated Caucasian HBV-monoinfected compensated cirrhotics were consecutively enrolled in a longitudinal (n=258) as well as in cross-sectional (n=111) studies. At baseline longitudinal cohort were: age 61 (43-77) year, 82% males, 88% HBeAg negative, 60% NUCs-experienced, BMI 25 (17-40) kg/m2, 12% diabetics, spleen length 11 (7-20) cm, 14% with esophageal varices, when transverse were: age 64 (51-77) year, 87% males, 93% HBeAg negative, 76% NUCs-experienced, BMI 25.1 (17-33) kg/m2, 19% diabetics, spleen length 11 (7-20) cm, 24% with esophageal varices. Seven SNPs mapping on genes above cited (rs7574865, rs4444903, rs17047200, rs4374383, rs6726639, rs738409 and rs641738) were analyzed by TaqMan genotyping assay.

Results: In the cross-sectional study, there were no significant difference between HCC patients (n=51) and controls (n=60) in rate of minor allele carriers of each SNPs investigated: 24% vs 27% for STAT4 (p=0.63), 52% vs 74% for EGF (p=0.09), 33% vs 30% for TLL1 (p=0.75), 75% vs 77% for MERTK (p=0.80), 57% vs 63% for MERTK2 (p=0.53), 52% vs 40% for PNPLA3 (p=0.33), 67% vs 62% for MBOAT7 (p=0.72). In univariate only correlation with HCC was spleen length (11 vs 11.5 cm, p=0.04). In the longitudinal cohort of 258 HBV patients followed on therapy for 123 (20-158) months, 45 (17%) patients developed an HCC after 56 (18-129) months. The 10-year cumulative HCC incidence was 20% (yearly rate 2.2%). The 10-year cumulative incidence of HCC was similar across different genetic variants while the only independent baseline predictors of HCC were and age (HR 1.09, 95%CI 1.0-1.1, p<0.001) and spleen length (HR 1.33, 95%CI 1.1-1.5, p<0.001).

Conclusion: In Caucasian HBV compensated cirrhotics treated with NUC, age and severity of portal hypertension, but not any of the 7 different genetic signatures, predicted the development of HCC in both longitudinal and cross-sectional studies.

P06-11 Circulating small-RNA signature evidenced a susceptibility to HCC occurence in DAA-treated patients

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Background and aims: The availability of an effective DAA-therapy for HCV infection has radically changed the possibilities of viral eradication, reaching up to 90% of SVR. Despite the expectations, recent studies raised concerns about an unexpected higher recurrence of HCC after DAA therapy. However, large cohort studies observed a reduced HCC risk in patients with SVR, compared to non-responders, remaining a residual risk of tumor development especially in cirrhotic subjects. Most studies so far pointed out the need of developing model-based evaluations of the risk of HCC in DAA-treated individuals using available biomarkers. In the present study, we explored the potential use of circulating Small-RNAs as serum surrogate biomarkers to identify patients at risk for HCC development.
Method: We examined the expression levels of the circulating small-non-coding RNA (sncRNA) in 10 matched patients with chronic HCV infection that underwent to DAA therapy. Microarray analysis was performed before (T0) and after 1 month of the DAA-therapy (T1). Patients were divided into two groups, patients with HCC (HCC+) or without HCC (HCC-) occurrence within 12 month after DAA therapy. sncRNAs discriminating HCC+ and HCC- patients were validated in 60 samples by means of RT-qPCR. We estimated the time-averaged difference of a given miRNA between HCC+ and HCC- patients using a bootstrapped random-effect generalized least square regression model (RE-GLS).
Results: miRNa are the most represented class of circulating sncRNAs in our samples. Mature miR-1207-5p, miR-1275, miR-3197, miR-4443, miR-3178, miR-483-5p, miR-4706, miR-4793-3p, miR-1246, miR-1180-3p, miR-1228-3p, miR-4329 and miR-4484 discriminated HCC+ from HCC- patients ($p < 0.05$). The validation phase identified miR-3197 as significantly different considering both disease and time, showing a lower expression in patients developing HCC. MiR-3197 significantly discriminates HCC+ from HCC- patients at T0 (AUC=0.75, 0.50-0.89, 95%CI, p= 0.004, sens =80%, spec=73%) and T1 (AUC=0.75 (0.48-0.89 95%CI, p=0.007, sens=86%, spec=73%). The mir-3197 was confirmed to be downregulated in tumoral tissue compared to the paired non-tumoral tissue.
Conclusion: The results suggest that patients are already committed to HCC occurrence before DAA therapy. MiR-3197 shows some potential for the identification of patients at risk of HCC during DAA treatments.

P06-12YI Therapy with DAA increases post-OLT survival but not the risk of recurrence in patients undergoing liver transplantation for HCV-related HCC

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Background and aims: The role of direct acting antivirals (DAA) for hepatitis C virus (HCV) in hepatocellular carcinoma (HCC) recurrence is a source of great debate. Current evidence is not able to determine whether DAA therapy either increases or decreases risk of recurrence. However, it has not been thoroughly investigated the role of DAA therapy in patients undergoing orthotopic liver transplantation (OLT) for HCC. As a post-OLT recurrence is an almost invariably deadly event, we aimed to compare the risk of recurrence and the overall survival (OS) of patients treated with DAA and obtaining sustained virological response (SVR) before OLT with an historical cohort of patients transplanted for HCC before the arrival of DAAs.

Method: We enrolled retrospectively patients transplanted in Bologna for HCC in a background of HCV-related cirrhosis divided in 48 patients in the DAA era (2015 to 2019) compared to 128 patients transplanted between 2003 and 2013, namely before arrival of DAA (historic group). We performed univariate analysis and then a multivariate Cox regression analysis including the significant variables aimed at identifying factors associated with time to recurrence and overall survival.

Results: Recurrence rate was 12.5% for the first group (mean time to recurrence 16.8 mo) and 20.3% in the historic group (mean time to recurrence 24.5 mo). OS was significantly different: 91.7% (DAA group) vs 60.6% (historic group). In the Cox analysis, active HCC at transplant, number of nodules and diameter of the largest nodule at transplant and LN10AFP at transplant were significantly associated with time to recurrence, as expected, whilst DAA treatment, microvascular invasion, diameter of the largest nodule at enlistment and LN10AFP at transplant were associated with OS. Analyzing the competing causes of death, most of the effects on the increased survival in the DAA cohort derived for the reduction of the risk of liver decompensation.

Conclusion: Post-OLT recurrence did not vary significantly between the historic and DAA-treated HCV cohorts. However overall survival was increased in the DAA group suggesting the their beneficial effect is mediated by the prevention of post-transplant HCV recurrence and subsequent long term liver decompensation.

P06-13YI Genetic risk factors of denovo hepatitis C-related hepatocellular carcinoma previously treated with direct acting antivirals

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Background and aims: The mechanism that underlies denovo development of hepatocellular carcinoma (HCC) following direct acting antiviral (DAAs) therapy for chronic hepatitis C is still under investigations. P53 is a tumor suppressor gene which plays a principal role in DNA repair, cell cycle arrest and apoptosis. Hepatocyte growth factor (HGF) is a cytokine with different functions such as liver regeneration. The aim of this work is to study P53 and HGF as possible predictor of denovo HCC following DAAs therapy in addition to AFP.

Method: This case control study included 83 patients with HCV-related liver cirrhosis divided into: Group 1: patients without HCC (n=25), Group 2 (n=25): patients with denovo HCC following DAAs therapy and achieving sustained virological response, Group 3: (n=33) patients with HCC without previous DAAs. P53 antibody and HGF were determined using a quantitative sandwich enzyme immunoassay technique (Cusabio Co, Houston, USA). The multiple logistic regression models were analysed to estimate the odds ratios (ORs) and 95% confidence intervals (95% CIs) of the association between biomarkers levels and susceptibility to de novo HCC following DAAs.

Results: Patients with denovo HCC following DAAs intake had significantly higher P53 levels than HCC patients without previous DAAs ($p < 0.0001$). The multiple logistic regression analysis showed that the increase in P53 levels was significantly associated with susceptibility to presence of de novo HCC following DAAs therapy ($P = 0.004$). The best overall Formula constructed by entering significant markers into the regression model. A three combined markers as developed = Combined markers= (1.22 + AFP X 0.002 + HGF X 0.001 + P53 X 0.001). The medians (percentiles) of combined three markers were 1.8 (1.-2.1) in CHC and 2.2 (2.0-2.9) in all HCC ($p < 0.00001$). The AUC of combined markers was greater than single markers. The AUC was 0.87 for differentiated HCC from liver cirrhosis; AUC was 0.91; for differentiated de novo HCC development after DAAs from CHC, AUC was 0.85 for differentiated HCC without undergoing DAAs from liver cirrhosis. However, the combined markers established an AUC value of 0.69, which was less than the value recorded from P53 individually (0.79)

Conclusion: P53 may serve as a diagnostic marker for HCV-related de novo HCC after undergoing DAA therapy

Figure:

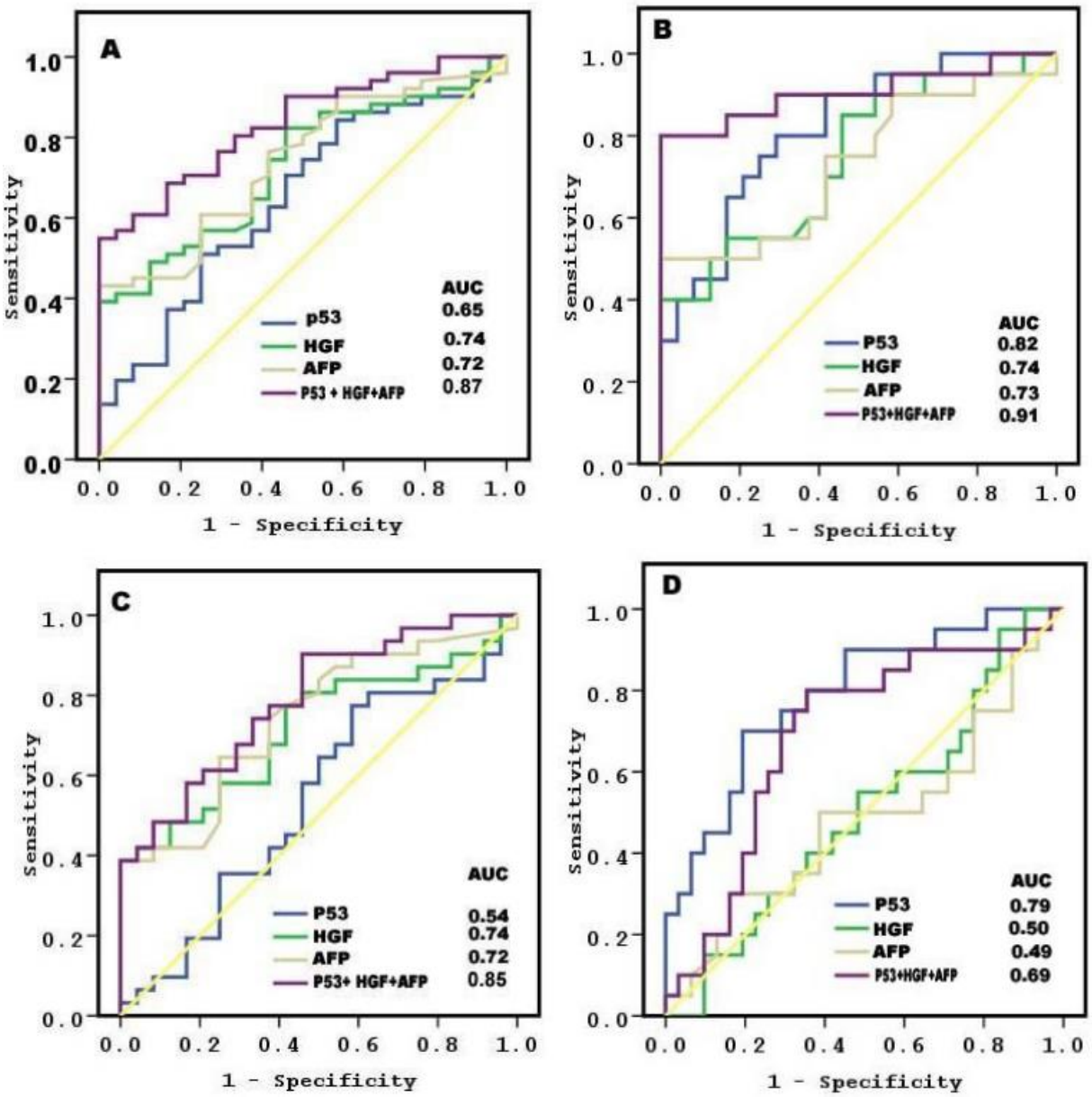

Fig.1. AUC of candidate markers P53, HGF and AFP.

P06-14YI Analysis of risk factors for survival and recurrence of hepatocellular carcinoma after liver transplantation

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Background and aims: Recurrence of hepatocellular carcinoma (HCC) after liver transplantation (LT) is an indicator of poor prognosis. Besides recognized risk factors for post-transplant HCC recurrence, other factors related to the biological behaviour of the tumour are scarce. The aim of this study was to identify the prognostic factors of the survival and HCC recurrence after LT.

Method: We retrospectively analyzed 260 HCC patients who underwent LT from 2002 to 2018. Median follow up was 41.5 months (0-165). Demographic, clinical, laboratory and HCC characteristics were analyzed.

Results: Patients were predominantly males with mean age 60.7 ± 8.04. Overall 187 (79%) patients had arterial hypertension (AH), 141 (59.2%) diabetes mellitus, 109 (47.4%) cholesterol above 5 mmol/L, 80 (35%) triglycerides (TG) above 1.7 mmol/L, 122 (53%) low-density lipoprotein cholesterol above 3 mmol/L, 69 (30%) high-density lipoprotein cholesterol below 1 mmol/L (HDL<1) and 47 (27%) had body mass index above 30 kg/m2. Most of them (48%) had alcohol-related liver disease. According to surgical specimen, 90 (36%) patients were not within Milan criteria, 91 (37%) had microvascular and 21 (8.5%) had macrovascular invasion (MaV). Median alpha fetoprotein (AFP) was 372 ± 1982.13 ng/mL. Most of patients had two immunosuppressive drugs (ID) (89%) and median waiting time on the list was 45.88 ± 119.18 days. Overall, 166 (63%) patients survived and 31 (12 %) patients had HCC recurrence with a median disease-free interval of 12.5 months. In multivariate analysis, HCC recurrence ($p < 0.001$) and HDL levels <1 ($p = 0.049$) were independently associated with worse survival after LT. Regarding HCC recurrence, higher levels of AFP ($p = 0.02$), presence of MaV ($p = 0.006$), larger total HCC diameter ($p = 0.002$) and more than one ID ($p = 0.006$) were independent risk factors for HCC recurrence. Using Cox proportional hazard regression, the independent risk factors for recurrence or mortality were AH (HR 0.5; 95% CI, 0.3 – 0.9), HDL<1 (HR 1.7; 95% CI, 1.0 – 2.9) and TG (HR 0.6; 95% CI, 0.4 – 0.9).

Conclusion: Our results indicate relationship between low HDL, high TG, AH and increased HCC recurrence as well as worse overall survival after LT. Better management of modifiable risk factors may reduce the incidence of HCC recurrence and improve patient survival after LT.

P06-15 Radiological response to trans-arterial chemoembolisation (TACE) determines outcome in patients with hepatocellular carcinoma (HCC)

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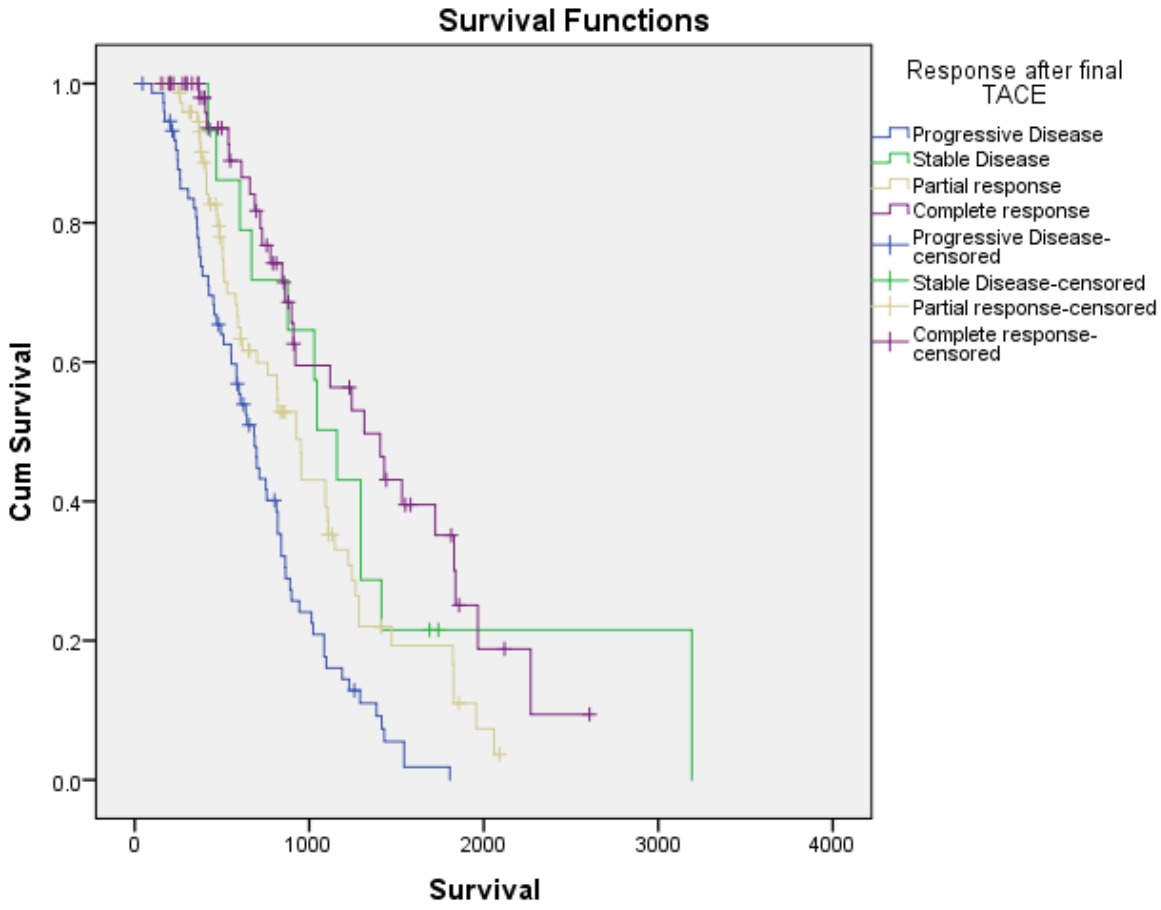
Background and aims: TACE is considered standard of care treatment for patients with Barcelona Clinic for Liver Cancer (BCLC) stage B HCC. Imaging post treatment determines further treatment offered, based on response. Radiological response is categorised as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD). Outcome can be predicted pre-TACE or following the first treatment based on a number of parameters. We wanted to demonstrate the survival differences based on radiological response to TACE alone when looking at patient outcome.

Method: All HCC patients treated with TACE at our regional centre since 2010 were included for analysis. Demographic details were obtained with proportion of cirrhotic patients, aetiology of liver disease, BCLC stage, Child Pugh Score and Hepatoma Arterial-embolisation Prognosis (HAP) score being calculated. Survival from diagnosis was calculated as well as the number of TACE procedures.

Results: 268 patients were identified as having HCC treated with TACE in this period. 84.7% male, 82.8% cirrhotic. Patients all had BCLC stage A or B disease and most (94.1%) Child Pugh A disease. Common causes of liver disease were: Alcohol (34.2%), NASH (28.8%) and Hepatitis C (10%). HAP score was: HAP A (38.2%), B (40.1%), C (19.5%) and D (2.2%). Overall median survival for all patients receiving TACE was 862 days (IQR: 766-957). There was a significant difference in survival based on response after initial TACE (CR: 1284, PR: 840, SD: 820 and PD 306 days, log rank $p<0.0001$) and response after final TACE (CR: 1316, PR: 926; SD 1159 and PD 684 days, log rank $p<0.0001$). There was no difference in survival based on Child Pugh stage ($p=0.700$) or if patients had BCLC A or B disease ($p=0.533$).

Conclusion: Our data suggests that patients' outcome following TACE for HCC relates to radiological response to treatment; those with CR having the longest survival and those with PD the shortest. Interestingly, those with PR and SD have similar outcomes based on response at first TACE and final TACE. Those with earlier stage disease (BCLC A) have similar outcomes more advanced disease (BCLC B) suggesting that treatment determines outcome rather than initial stage of disease.

Figure:


Survival Functions
Cum Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
1000
2000
3000
4000
Survival
Response after final TACE
Progressive Disease
Stable Disease
Partial response
Complete response
Progressive Disease-censored
Stable Disease-censored
Partial response-censored
Complete response-censored

P06-16YI miR-34a is activated in NAFLD and NASH-associated HCC, and correlates with key disease hallmarks

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) pathogenesis and progression to hepatocellular carcinoma (HCC) remains incompletely understood. We aimed to elucidate whether microRNA(miR)-34a overexpression represents a universal event in different diet-induced NAFLD animal models, as well as its association with key histological, clinical and biochemical features in human non-alcoholic steatohepatitis (NASH) and NASH-associated HCC.

Method: C57BL6 mice were fed five different NAFLD-inducing diets, namely a (1) methionine and choline-deficient diet for 2 and 8 weeks, (2) high-fat choline-deficient diet for 14 weeks, (3) high fat 2% cholesterol diet for 25 weeks, (4) high-fat calorie-rich diet with fructose and glucose supplementation for 16 weeks, and (5) choline-deficient amino acid-defined diet for 32 weeks. Liver miR-34a expression was evaluated by qPCR and in human biopsies obtained from 165 NAFLD patients (SS cohort) characterized at the histological and metabolic levels (NAS≤2: n=26; NAS 3 to 4: n=62; NAS≥4: n=77). miRNA sequencing (miRseq) was used to assess the miR-34a expression in resected samples from 19 NASH-associated HCC patients (TCGA cohort) and compared with surrounding liver tissue (n=50).

Results: Mice fed the five different diets developed various degrees of steatosis, NASH and fibrosis, including differences in weight gains and insulin resistance. Regardless of the model, liver miR-34a expression levels were significantly increased in all diseased mice compared with control diet-fed animals (at least $p<0.05$ for all). In NAFLD patients, liver miR-34a expression was found to progressively increase with steatosis, lobular inflammation and the NAS score (at least $p<0.05$ for all). miR-34a expression levels were the most augmented in patients with advanced fibrosis, including patients with concomitant diabetes, arterial hypertension and cholelithiasis (at least $p<0.05$ for all). In addition, univariate analysis indicated that liver miR-34a expression positively correlates with histological features (steatosis, lobular inflammation and fibrosis), serum hepatic ALT and AST, hepatic triglyceride content and age. Finally, miR-34a levels were strikingly elevated in the tumour tissue of patients with NASH-associated HCC compared with matched surrounding livers ($p<0.01$).

Conclusion: In conclusion, activation of miR-34a may be a key event governing NAFLD to HCC progression, correlating with several specific, well-characterized human disease hallmarks. (Gilead Sciences International - Research Scholars Program in Liver Diseases; PTDC/MED-PAT/31882/2017 and SFRH/BD/104160/2014, FCT, Portugal; CENIE/0348_CIE_6_E/OLD-HEPAMARKER, INTERREG, POCTEP).

P06-17YI A novel significance of apurinic/apyrimidinic endonuclease/redox effector factor 1 protein in hepatic cancer stem cells

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Background and aims: The human apurinic/apyrimidinic endonuclease/redox effector factor 1 (APE1/Ref-1) is a multifunctional protein with important DNA repair and redox capabilities, also in the regulation of genes involved in chemoresistance and cancer progression. Recently, cancer stem cells (CSC) have emerged as a major cancer topic to be the origin of cancer, including hepatocellular carcinoma (HCC). Since CSC is chemoresistant through its function in DNA repair, it is logical to suggest that APE1/Ref-1 is also involved in CSC activities. Here we aim to investigate a direct correlation between APE1/Ref-1 and hepatic CSC.
Method: Hepatic cell lines IHH, HepG2, and Huh7 were employed as *in vitro* model. Manipulation of cells populations expressing various CSC markers including the CD13/ANPEP, CD133/PROM-1, and EpCAM were performed by RNA-silencing and CSC isolation using magnetic and flow-activated-cell-sorting. All cells were also treated with Ape1 inhibitor E3330 with concentration ranging from 10 to 125 uM. Cytotoxicity was assessed by MTT dye reduction assay. Changes of CSC biomarkers were assessed by RTqPCR and flow cytometry for mRNA expression and CSC quantification, respectively. Data *in vitro* was validated *in vivo* by immunohistostaining of both APE1/Ref-1 and CD13 protein on paraffinated HCC clinical slides.
Results: APE1 mRNA expression was significantly increased and maintained in CD13-silenced cells in a dose-dependent manner. APE1 expression was also higher in separated $CD13^{low}$ and $CD133^{low}$ compared to $CD13^{high}$ and $CD133^{high}$ CSC subpopulations. High APE1 was accompanied by high miR-1246. Gradual increase of E3330 concentration induced the number of CD13, CD133, and EpCAM CSC populations ($p<0.05$) together mRNA up-regulations. From *in vivo* data, immunostaining on multiple HCC slices showed that APE1 positive staining was observed in CD13 negative/low staining area.
Conclusion: We confirmed that APE1/Ref1 was differentially expressed in hepatic CSC populations, probably related to cellular hierarchical differentiation.

P06-18 Empowerment of liver cancer patients for an improved management of post-embolization syndrome: Impact of a Nurses Educational program

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Background and aims: Chemoembolization (TACE) is the recommended treatment for hepatocellular carcinoma in the BCLC intermediate stage classification. The most frequent adverse events are fever, pain, nausea and fatigue known as 'post-embolization syndrome (PES)'. PES can cause anxiety and prompt consultations, unscheduled outpatient visits and emergency room attendance. The study evaluates how the BCLC Nurses Educational Program (BCLC-NEP) encourages patient autonomy after TACE to optimally manage PES.

Method: We retrospectively analyzed prospective data of all BCLC TACE-treated patients from Feb/2014 to Feb/2017. The program includes three key nurse visits: an outpatient visit before treatment, an inpatient visit post-procedure, and a 1-week-postTACE outpatient visit and phone consultations on request. This study analyzed symptoms reported after hospital discharge for the first two TACE sessions, and registered who resolved the first PES complaint reported by each patient.

Results: We reviewed 104 TACE-candidates: 92 TACE-treated patients who received at least the first TACE [median age 69 (range; 43-85), 85% male, (BCLC-A 46.7%, BCLC-B 53.3% and Child Pugh A (92.4%)]; 63 patients who received a 2nd TACE.

Seventy-nine patients (75.9%) reported PES after the first TACE, 52 (65.8%) were able to manage symptoms autonomously, 17 (21.5%) received nurse intervention and 8 (10.1%) required physician intervention. Patient interventions followed the BCLC-NEP recommendations regarding self-monitoring (50%), medication use (38.5) and other issues (7.6%). Only two patients (3.9%) attended the emergency department due to fever and pain. The main nurse intervention after TACE involved education and empowerment (88.2%).

After the second TACE, 50 patients (79.3%) reported PES, 39 (78%) were able to resolve the first sign/symptoms autonomously, 9 (18%) needed nurse intervention and 2 (4%) required physician intervention. Resolution of patient issues was achieved by following instructions regarding self-monitoring 46.2% and drug intake 53.8%. The main role of nurses after the second TACE was education (88.9%).

Conclusion: The BCLC-NEP for TACE patients promotes patient autonomy in PES management. The impact of the program proved more noticeable after the second TACE when patients had more experience with treatment and PES management (78%). This study reflects the extent to which the professional skills of nurses has an impact on the management of patients with liver cancer.

P06-19YI SImBA: A user-friendly high throughput tool for spheroid invasion analysis of hepatocellular carcinoma cell lines

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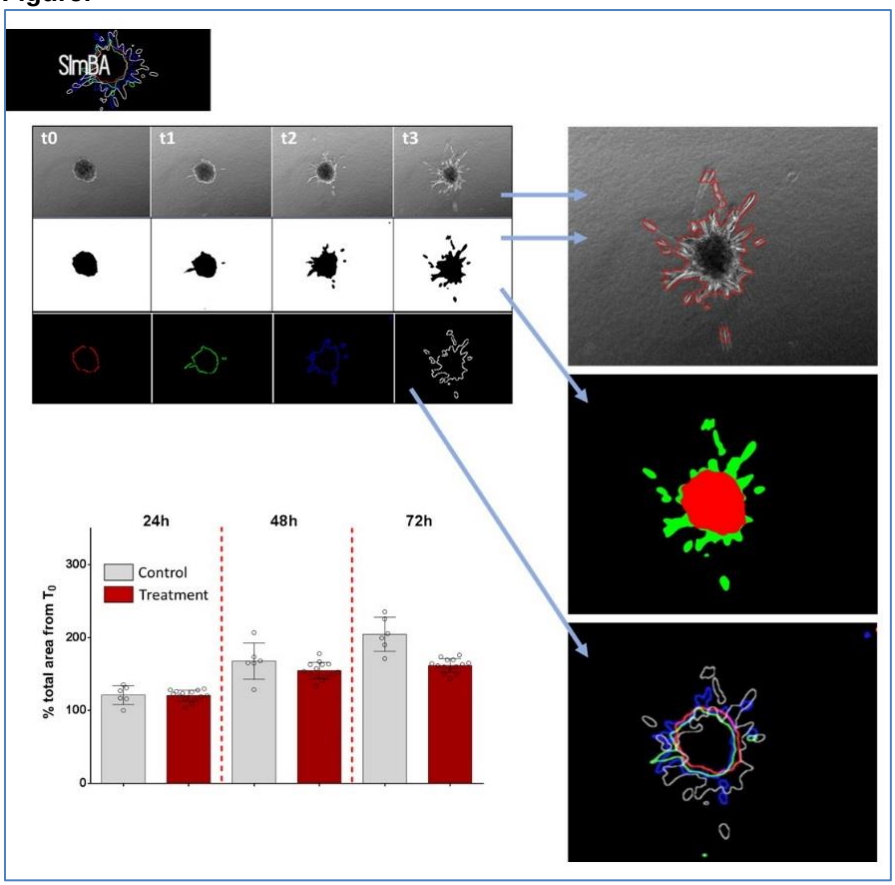
Background and aims: Due to the complex pathology of hepatocellular carcinoma (HCC) and its asymptomatic early stages, intrahepatic invasion and extrahepatic metastasis to a.o. the lung and abdominal lymph nodes are often already present at diagnosis. The evaluation of treatment effects on HCC invasion in *in vivo* models is currently far from evident[A] and not feasible for high-throughput drug screening. 3D *in vitro* assays such as the spheroid invasion assay (SIA) have already proven their physiological relevance in different cancer types[B] and have been applied in experimental HCC[C]. In order to use SIA in high-throughput screening of HCC drugs, methods for fast and in depth data analysis are required. Accordingly, we developed SImBA (Spheroid Image Batch Analysis), a FIJI-based software tool which allows to perform high-throughput image segmentation, visualization and quantification of invasive spheroids.
Method: Spheroids of two HCC cell lines (HEP3B and SNU423) were embedded in a 3D collagen Type I matrix. Phase contrast microscopy images were recorded at different time points to evaluate the invasive process of control and treated spheroids. Since FIJI is commonly used free software[D], SImBA has been written as an open-source FIJI macro which ensures high user-friendliness and easy customization by the user. SImBA allows batch image processing independent of data set size and is applicable on images of variable and low contrast.
Results: Since SImBA is able to visualize and quantify the invasion data in multiple ways (using total spheroid area, spheroid outlines, area overlaps, montages and a derived invasion index), it allows to robustly demonstrate the effects of various drugs compared to control treatment. Importantly, effects on invasion and cytotoxicity can be linked using SImBA when fluorescent images of cytotoxicity from e.g. Sytox green staining are recorded
Conclusion: We created a highly functional, easy to use free software tool for the quantitative analysis of high-throughput spheroid invasion and visualization of the invasive capacity of hepatic cancer cell lines. This will contribute to future drug screening in HCC using either 3D SIA or related assays using organoids.

Figure:



A: N P Santos, et al, Tumor Biology, 2017 Mar
B: Nath S, et al, Pharmacol Ther. 2016 Jul
C: Khawar IA, et al, Neoplasia, 2018 Aug
D: https://fiji.sc/

P06-20 Involvement of the glycan-binding protein galectin-1 in hepatocellular carcinoma cell drug resistance

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Background and aims: Hepatocellular carcinoma (HCC) is characterized by a high resistance to chemotherapy. P-glycoprotein (Pgp) is an ATP-dependent drug efflux pump and its overexpression in HCC is associated with a decrease in intracellular drug concentration, leading to chemotherapeutic tolerance. Galectin-1 (Gal1), a β-galactoside-binding protein, is overexpressed in HCC and it is related to tumor aggressiveness. Recent studies suggest that Gal1 may have a role in HCC chemoresistance. Our aim was to investigate the molecular basis of Gal1-mediated chemoresistance in HCC cells.

Methods: We stably transfected human HCC HepG2 cells to overexpress (HepG2Gal1) or silence (shRNA) Gal1 expression, with the corresponding control transfections. To investigate Gal1 involvement in HCC chemoresistance *in vivo*, we evaluated whether HepG2Gal1 cells could generate doxorubicin (DOX)-resistant tumors in immunodeficient NSG mice. MTT assay was performed to determine if different levels of Gal1 expression affect viability in cells exposed to DOX or sorafenib. To elucidate the involved mechanism, the amount of intracellular DOX was determined using fluorescence techniques. The effect of Gal1 on Pgp expression was studied by immunoblotting.

Results: We observed a decrease in the volume of control-derived tumors treated with DOX (4.5 mg/kg i.v. once a week, 3 weeks) compared with HepG2Gal1-derived treated tumors (0.43±0.03cm^3 vs 1.39±0.38cm^3, p<0.05). By comparison of half maximal inhibitory concentration values we found that Gal1 overexpression significantly protected HepG2 cells from DOX (1.31µM vs control cells: 0.81µM) and sorafenib (32.25µM vs control cells: 13.36µM) exposure, while silencing Gal1 sensitized cells to drug cytotoxic effects. Intracellular DOX concentration decreased in HepG2Gal1 cells versus control cells (3.1±0.5 vs 4.5±0.3 pmol/µg total protein, 6h-treatment, p<0.01). Gal1 knockdown induced the opposite effect (p<0.01). Gal1 overexpression increased Pgp protein levels in a PI3K-dependent manner (p<0.05). Co-incubation of HepG2Gal1 cells with 2µM DOX and 20µM Pgp inhibitor verapamil diminished cell viability compared with cells incubated only with DOX (p<0.05). Similar results were obtained by siRNA-mediated Pgp knockdown (p<0.05).

Conclusion: Gal1 protects HCC HepG2 cells from DOX- and sorafenib-induced cell death. Also, Gal1-overexpressing cells accumulate less intracellular DOX. Moreover, P-gp is involved in Gal1-induced resistance to DOX.

P06-21YI Transcriptome profiling of liver biopsies before antiviral treatment start can predict HCC development 8.3 years before clinical diagnosis in chronic hepatitis B and C patients

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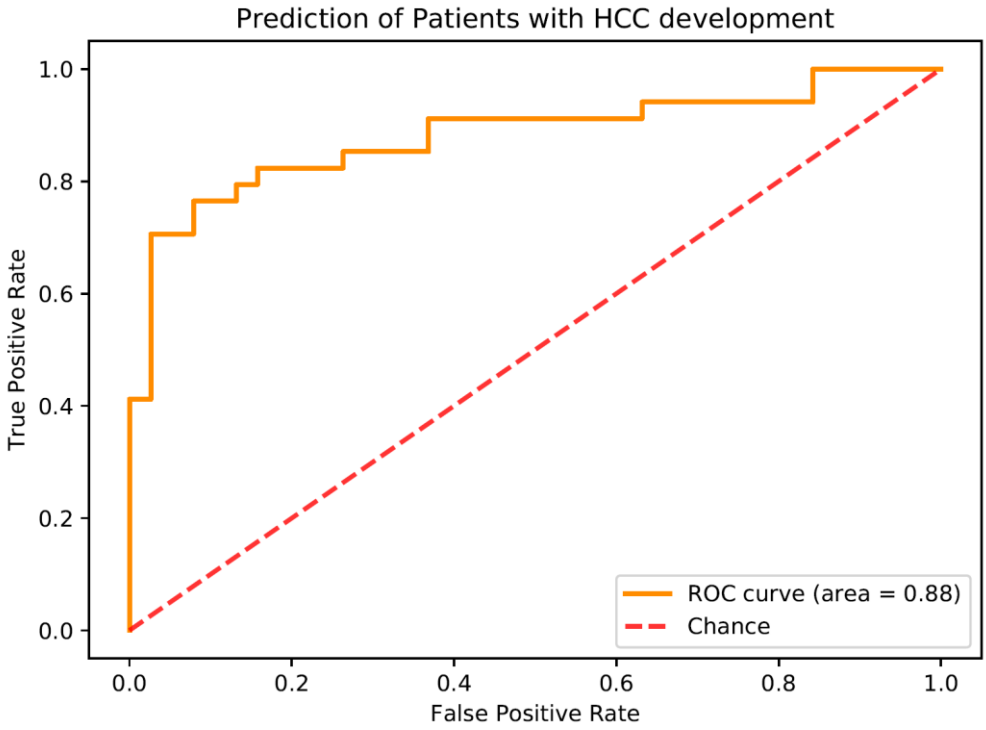
Background and aims: An accurate prediction of Hepatocellular Carcinoma (HCC) development in Chronic Hepatitis B (CHB) and C (CHC) patients is currently impossible. In this study we explored pre-antiviral treatment liver transcriptome profiles of CHB and CHC patients with and without HCC development during long-term follow-up and investigated their potential to predict future HCC development.

Method: HCC developing cases (n = 34) were identified through retrospective chart review of all CHB and CHC patients with an available pre-antiviral treatment liver biopsy from 5 large Hepatology clinics. Cases were split in 4 subgroups based on infecting virus (HBV/HCV) and cirrhosis status (yes/no) at baseline liver biopsy. Each subgroup of cases was matched for different demographic (e.g. gender and age at biopsy) and clinical (e.g. cirrhosis at biopsy and infecting virus) factors to a group of controls without HCC development during an equal or longer follow-up time. RNA derived from baseline biopsies (total n = 72) was sequenced. Differentially Expressed Genes (DEG; FC > 1.5 and q < 0.2) were called in each subgroup and a random forest classifier was trained to predict HCC development.

Results: The total cohort consisted of 72 patients, of whom 34 developed a HCC at a median of 8.3 years after liver biopsy. Despite perfect matching for clinical and demographic characteristics, at least 452 DEG were found between cases and controls in each subgroup. Among the top 20 up- and down-regulated genes in each subgroup, 40-75 % has previously been linked to oncogenesis, underlining the biological relevance. Ingenuity Pathway Analysis showed an enrichment for the “Wnt-bèta catenin signaling” pathway in the cirrhotic CHB group and the “molecular mechanisms of cancer” pathway in the non-cirrhotic CHC group. These results strongly suggest a genetic imprint for HCC development several years before clinical diagnosis. A random forest classifier tested with leave-one-out-cross-validation was able to predict HCC development with an accuracy of 84.7 % (See Figure), a Negative Predictive Value of 92.1 % and a Positive Predictive Value of 75.8 % based on the subgroup and baseline expression levels of 20 genes, of whom several have previously been linked to hepatocarcinogenesis.

Conclusion: Pre-antiviral treatment liver biopsies of chronic hepatitis B and C patients show a genetic imprint for future HCC development that allows to accurately predict HCC development 8.3 years before clinical diagnosis.

Figure:


Prediction of Patients with HCC development
True Positive Rate
False Positive Rate
0.0
0.2
0.4
0.6
0.8
1.0
ROC curve (area = 0.88)
Chance

P06-22YI ZEB1 promotes cholangiocarcinoma progression through tumor dedifferentiation and paracrine signalling between tumor cells and cancer-associated fibroblasts

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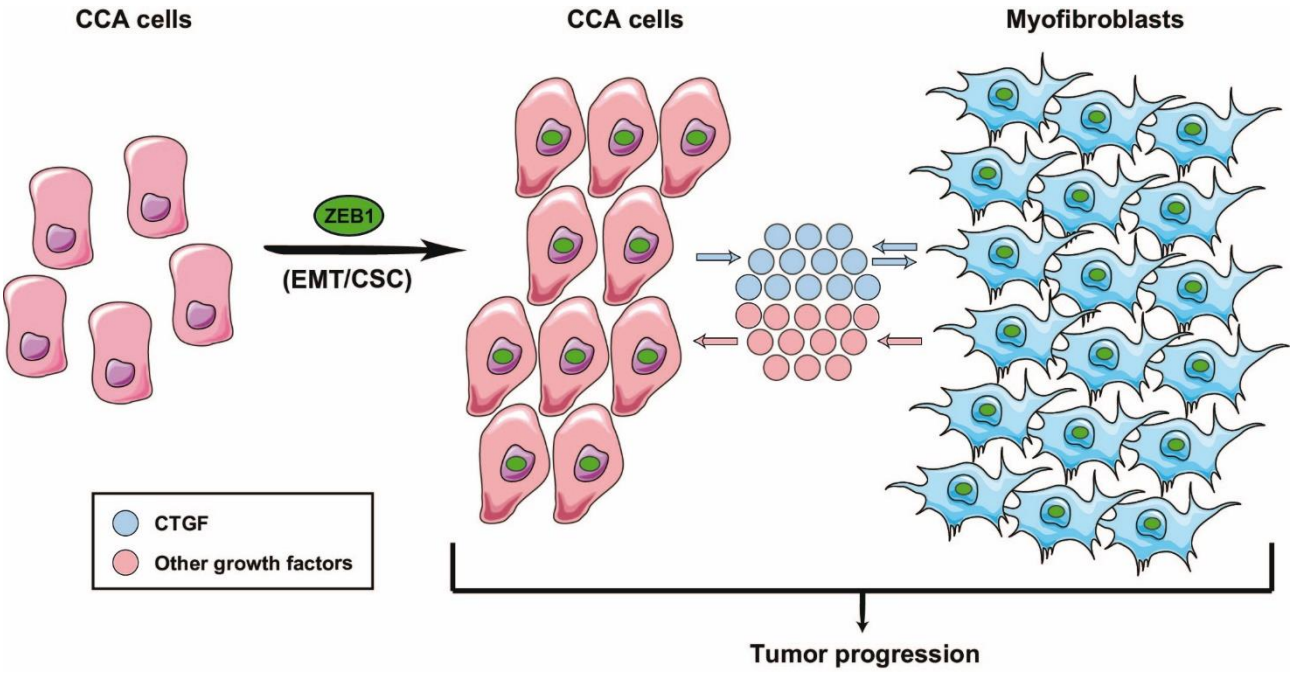
Background and aims: Cholangiocarcinoma (CCA) arises from biliary epithelial cells and has poor prognosis due to its late clinical presentation and the lack of effective non-surgical treatment. CCA is characterized by a prominent fibrous stroma mainly composed of cancer-associated fibroblasts (CAF). ZEB1 is a transcription factor expressed by tumor and stromal cells including CAF that contributes to the acquisition of metastatic and stem cell properties. Recently, ZEB1 has been associated with poor prognosis in CCA. Yet, the regulatory functions that ZEB1 exerts in CCA are poorly defined.

Method: Correlations of ZEB1 expression with selected markers in human CCA, were investigated by transcriptomic analyses of whole tumor or laser microdissected stroma and by immunohistochemistry. Viral infection was used to generate gain/loss of function models in human CCA cell lines and liver myofibroblasts (LMF), i.e. immortalized activated hepatic stellate cells, as a model of CAF. Conditioned media (CM) was used to examine tumor-stroma communication. In vivo experiments were performed using a xenograft tumor model in immunodeficient mice.
Results: ZEB1 was expressed in tumor cells in 20% of human CCA and its expression was associated with poor differentiated tumors ($p<0.05$). In vitro, ZEB1 promoted the acquisition of EMT/CSC traits in tumor cells along with increased migration and spheroid formation. In vivo, CCA cells that expressed ZEB1 formed larger tumors with higher stromal content. Interestingly, we found an increased expression of the fibrotic marker CTGF in tumor cells and in human and mouse CCA that correlated with ZEB1 expression, suggesting a role of CTGF in the development of desmoplastic microenvironment. In vitro, CM from CCA tumor cells overexpressing ZEB1 or CTGF induced LMF proliferation. Moreover, ZEB1 was found to be expressed by alpha-SMA positive CAF in human CCA. At mRNA level, ZEB1 correlated with alpha-SMA, COL4A1, TGF-beta and CTGF in LMF and in human and mouse CCA stroma. Co-injection of tumor cells plus ZEB1-expressing LMF generated larger tumors than tumor cells plus ZEB1-invalidated LMF. Crosstalk experiments showed that CM from ZEB1-expressing LMF increased tumor cell viability as compared to CM from ZEB1-invalidated LMF.
Conclusion: ZEB1 plays a key role in CCA progression by regulating tumor cell-LMF cross-communication, leading to tumor dedifferentiation and CAF activation.

Figure:


CCA cells
CCA cells
Myofibroblasts
ZEB1
(EMT/CSC)
CTGF
Other growth factors
Tumor progression

P07-01YI Intratumoral EpCAM-positive cancer stem cell heterogeneity in patients with hepatocellular carcinoma and its impact on clinical outcome

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Background and aims: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death. The genomic and histopathological heterogeneity among and within HCC tumours is of increasing interest. Dismal prognosis is often linked to invasiveness and metastatic potential of HCC harbouring cancer stem cell (CSC)-features (e.g. EpCAM-expression). However, knowledge on intratumoral EpCAM-expression and its impact on recurrence after curative-intended resection remains limited. This study aimed to investigate the spatial heterogeneity of EpCAM-expression within early HCC, and to identify its potential impact for the risk stratification of recurrence.

Method: We screened our biobank for suitable tissue from patients undergoing liver resection or transplantation between 2011 and 2017 to design a tissue microarray (TMA). Tumour specimens for TMA construction were sampled at multiple locations (n=3-8). EpCAM-positivity was assessed for intensity and proportion to derive a score dividing three groups: EpCAM-negative (E-/-), heterogeneous-positive (E-/+), homogeneous-positive (E+/+). EpCAM score was correlated with the major clinical primary endpoints time to recurrence (TTR) and recurrence free survival (RFS)

Results: Overall we included 341 tumour spots from 75 patients (77% male, median age 66 years, liver cirrhosis 40.3%, liver fibrosis 34.3%). Aetiology consisted of alcoholic liver disease in 24.1%, NASH 16.5%, HBV 14.3%, HCV 17.6% and others 27.5%, representing a typical Western cohort. EpCAM score resulted in 32 E-/-, 36 E-/+, and 7 E+/+. E+/+ patients experiencing significantly shorter TTR and RFS compared to E-/- and E-/+ patients (TTR 5 vs. 19 vs. 19 months, p=0.03; RFS 5 vs. 14 vs. 15 months, p=0.028, respectively). Interestingly, homogeneous EpCAM-positivity correlated with AFP levels >400 ng/ml, p=0.034. A single spot had a positive predictive value for homogenous EpCAM-positivity of 45.7%, a negative predictive value of 100%, a specificity of 87.4% and a sensitivity of 100%.

Conclusion: Intratumoral EpCAM-expression has a high spatial heterogeneity. Only homogeneous positivity is significantly correlated with worse outcome, identifying patients in urgent need for adjuvant treatment. Additionally, our study demonstrates the importance of multiple sampling, which should be considered to identify patients for targeted treatment.

P07-02YI High rate of HBV S-integrated human ESPL1 fusion gene is detected in HBV-related liver cancer patients: A Chinese case-control study

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Background and aims: It has been shown that the integration of hepatitis B virus (HBV) gene into the host genome is a high-risk factor for hepatocellular carcinoma (HCC) development. However, the relationship between HBV S-integrated human Extra Spindle Pole Bodies Like 1 (ESPL1) gene and HCC is unknown. This study was designed to detect HBV S-integrated human ESPL1 fusion gene in HCC patients for potentially developing this fusion gene as a biomarker for HCC diagnosis.
Method: Nineteen and 70 chronic hepatitis B (CHB) patients were recruited to the experimental and control groups, respectively, and both groups underwent an effective nucleoside/nucleotide analogs (NUCs) therapy and follow-up for HCC occurrence for up to 11 years. HCC tissues were obtained by surgical resection from the experimental group, while liver tissues were collected by liver biopsy in the control group prior to NUCs treatment. Alu polymerase chain reaction (PCR) was used to assess HBV S gene integration in the liver tissues from both groups. HBV S-integrated human ESPL1 fusion gene was then detected in the patients with HBV S gene integration by using the gene database.
Results: We observed that all CHB patients in the experimental group developed HCC, whereas no HCC was diagnosed in the control group. HBV S gene integration was identified in 12 out of 19 HCC tissues in the experimental group with a detectable rate of 63.2% (12/19), which was significantly greater than that of 15.7% (11/70) in the control group (P=0.000). We further showed that HBV S-integrated human ESPL1 fusion gene was detected in 8 patients with a rate of 66.7% (8/12) among the 12 HCC patients with HBV S gene integration in the experimental group, whereas the fusion gene was not detectable in any one among the CHB patients in the control group (P=0.001) (Fig 1).
Conclusion: This research demonstrates a high detectable rate of HBV S-integrated human ESPL1 fusion gene in HBV-related HCC patients and shows that this fusion gene is associated with HCC development in CHB patients. These findings suggest that HBV S-integrated human ESPL1 fusion gene may potentially serve as a biomarker for early detection of HCC in the HBV-infected populations.

Figure:

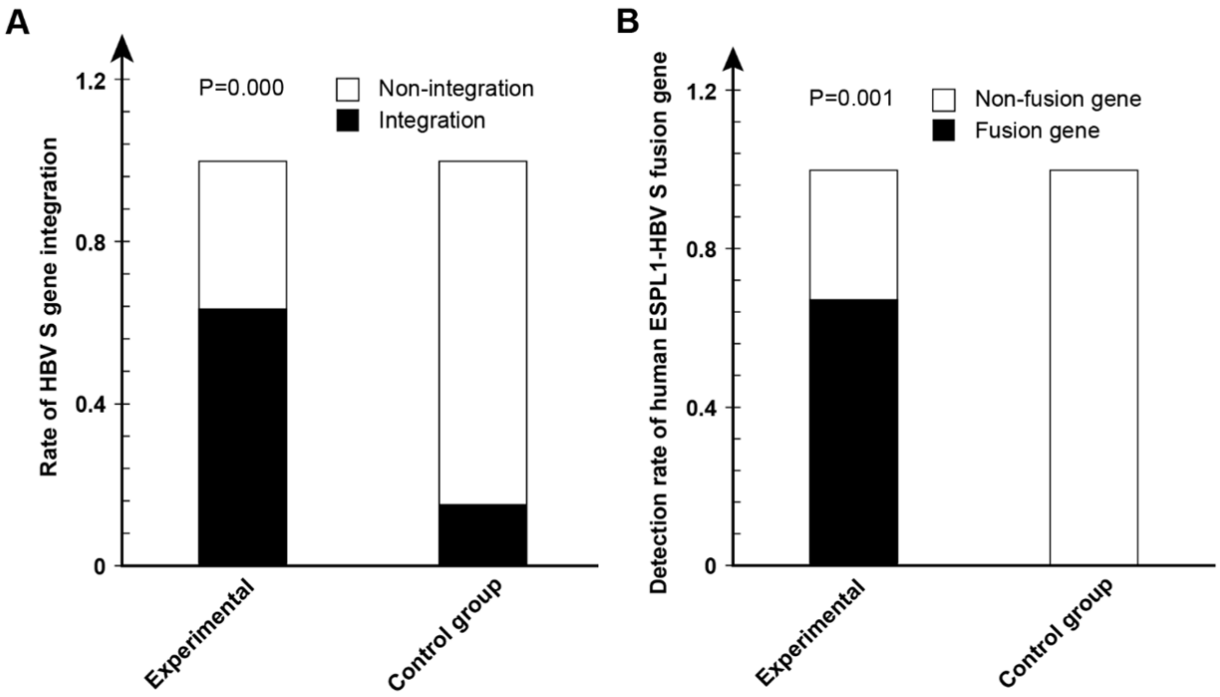

Figure 1. Detection rate of HBV S gene integration and human ESPL1-HBV fusion gene in the experimental and control groups. (A) The comparison of HBV S gene integration in both the experimental and control groups by chi-square test of four-fold table. Our data indicate that HBV S gene integration rate is significantly higher in the experimental group than that in the control group (P=0.000). (B) The comparison of the detection rate of the human ESPL1-HBV S fusion gene between the experimental and control groups by Fisher's exact probability. The data show that the detection rate of human ESPL1-HBV S fusion gene is 66.7% in the experimental group as compared with 0% in the control group (P=0.001).

P07-03 Urinary epidermal growth factor-related transforming growth factors and serum alpha-fetoprotein as tumour markers of hepatocellular carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) is one of the most lethal form of malignancies. The poor prognosis has been attributed to its insidious onset, late presentation at diagnosis and lack of a prudent diagnostic method for timely diagnosis. Recent insights indicate that peptide growth factors are important in the tumour cell growth. Previously, we reported overexpression of epidermal growth factor-related transforming growth factors (EGFRTGFs) in the urine of patients with HCC. Although serum alpha-fetoprotein (AFP) is still the most used tumour markers for HCC, its diagnostic accuracy is disappointed. This case-control study aimed to assess the diagnostic application of urinary EGFRTGFs in HCC.

Method: The studying population included 96 pairs of cirrhotic patients with HCC and those without and 60 healthy controls. Both urinary EGFRTGFs and serum AFP were measured with radioreceptor assay and radioimmunoassay, respectively. Multivariate analysis was used to adjust the confounding effects of sex, age, status of HBsAg and antiHCV.and conventional liver function tests. Diagnostic performance and the optimal cut-off values were determined with receiver operating characteristic (ROC) curves.

Results: The levels of EGFRTGFs and AFP in patients with HCC were higher than those in patients with cirrhosis alone or healthy controls (each p = 0.0001, respectively). Multivariate analysis indicated that EGFRTGFs (odds ratios (OR) 1.10; 95% confidence interval (CI), 1.05 - 1.15) and AFP (OR, 1.03; 95% CI, 1.01 – 1.06) were independent risk factors for HCC. They were associated, in a dose–related fashion, with an increased risk for HCC.The optimal cut-off values determined with ROC curves were 32 ug/g creatinine for EGFRTGFs and 100 ng/ml for AFP, respectively. The area under ROC curve (AUC), sensitivity, specificity, diagnostic accuracy, and positive predictive value for EGFRTGF and AFP were 0.76, 51%, 100%, 75.5%, and 94.4%, vs 0.75, 48.9%, 100%, 74.4% and 100%, respectively. Determination of both markers in parallel significantly increase the AUC (0.88), sensitivity (77.0%) and accuracy (88.0%), with a little decrease specificity (98.9%) and PPV (98.6%). Among 35 HCC patients with AFP ≤ 20 ng/ml, the calculated AUC, sensitivity, specificity, PPV and positive likelihood ratio were 0.78, 62.9%, 99.0 %, 95.7% and 62.9.

Conclusion: Urinary EGFRTGFs and serum AFP are complimentary tumor markers of HCC, particularly in patients with low AFP production.

P07-04 Prognostic value of desmoplastic stroma in intrahepatic cholangiocarcinomas

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Background and aims: Intrahepatic cholangiocarcinomas (ICC) are primary tumors of the liver characterized by the presence of a desmoplastic stroma. Its prognostic role is still an open question. In malignancies, tumor stroma may be benefit, acting as a barrier against cancer diffusion or pejorative, by supporting the tumoral cells. The aim of the present study was to evaluate the prognostic value of stromal compartment in ICC through a multiparametric morphological analysis

Method: Forty-nine patients (61 years) with ICC surgically resected were included. For all cases, tumor paraffin blocks of ICC were selected. Stromal area and Cancer-Associated Fibroblats (CAF) number, were automatically quantified on Sirius red staining and alpha smooth muscle actin expression, respectively. Activated stroma index (ASI) was calculated as the ratio of CAF number and stromal area. Collagen fiber reticulation properties were analysed using second harmonic generation imaging.

Results: High stromal area was inversely correlated with vascular invasion (62.5% vs 95.7%, p=0.006) and positively correlated with well differentiated tumors (60% vs 12.5%, p=0.001). Patients with high stromal area had a better disease free survival (DFS) than patients with low stromal area (60 % vs 10 %, p=0.077). Low ASI was correlated with a better DFS (60% vs 10%, p=0.05). High collagen reticulation index was correlated with a worsened overall survival (42% vs NR, p=0.026).

Conclusion: Desmoplastic stroma seems to exert protective effect in patients with ICC. Stromal collagen reticulation may provide additional clinically relevant information in malignancies.

P07-05 Clinical significance of serum albumin functional parameters for diagnosis of hepatocellular carcinoma

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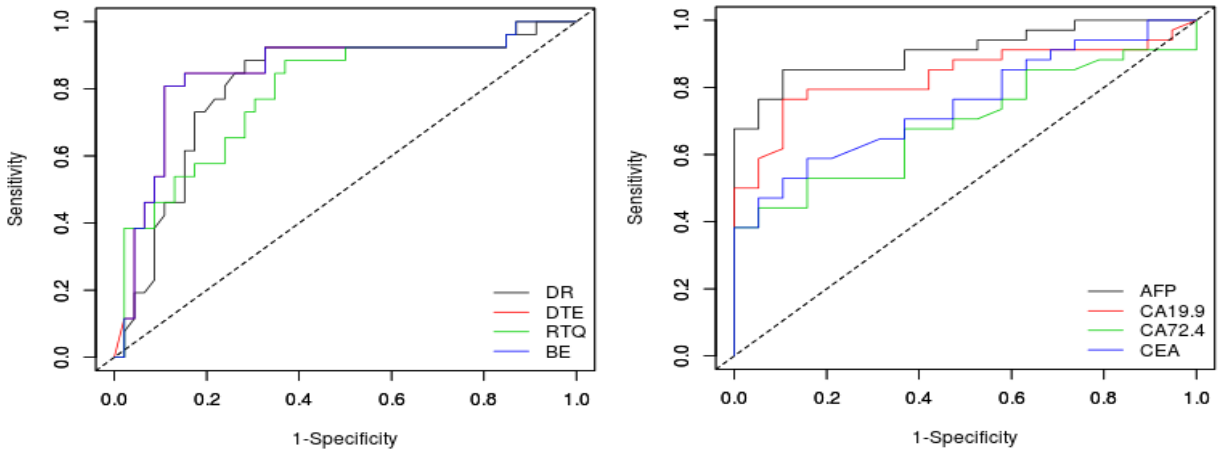
Background and aims: Albumin plays an important role in detoxification of organisms. Albumin is the main carrier protein in
the circulatory system that provides binding of hydrophobic toxins and their transfer to hepatocytes. It seems likely that in patients with hepatocellular carcinoma (HCC) there can be a specific liver function impairment versus patients with chronic liver diseases (CLD), which can be assessed using functional parameters of albumin molecules. The objective of this work was to assess the diagnostic feasibility of serum albumin functional activity and molecular conformation by electron paramagnetic resonance for diagnosis of HCC.
Method: Blood samples were obtained from 46 patients with HCC and 26 patients with chronic liver diseases
admitted to the Dufle Specialist Hospital (Hodan District, Benadir, Somalia). Parameters of albumin functional activity (DTE detoxification efficiency, BE binding ability, RTQ transport efficiency) and conformation of albumin molecules (DR) were measured by electron paramagnetic resonance using EPR AXM-09 Laboratory Analyzer and ATA-test-C-80 reagents set (Albutran, Belarus). Clinical chemistry parameters (ALT, AST, creatinine, CEA, CA19-9, CA72-4, AFP) were measured on a Cobas-6000 modular system (Roche, Germany).
Results: In the HCC patients DR was significantly changed comparing to the CLD patients: median DR values
were -0.35 for HCC patients and 0.64 for CLD ($p<0.001$). In addition, the average value of the DTE in the HCC was decreased to 43.9%, while in CLD patients it was 100.6% (median 34.3% and 95.4% respectively, $p<0.001$). Other parameters of albumin functionality also differ significantly in HCC and CLD groups: BE mean values were 55.5% vs. 98.6% (median 51.2% vs. 97.5% respectively, $p<0.001$), mean DTE values were 52.6% vs. 75.3% (median was 53.0% vs. 81.6% respectively, $p<0.001$).
At the same time, the diagnostic informativity of the DTE albumin parameter (AUC = 0.854, SE = 84.6%, SP = 84.6%) was higher than for CA19-9 (AUC = 0.836, SE = 78.9%, SP = 79.4%) and CEA (AUC = 0.752, SE = 63.2%, SP = 70.6%). Maximum informativity was observed for AFP: AUC = 0.874, SE = 89.5%, SP = 85.3% (all $p<0.05$).
Conclusion: The obtained results show specific changes in albumin functional parameters of HCC patients.
Transport function parameters and molecular conformation of albumin can be used for HCC laboratory diagnosis with a sensitivity and specificity of 80% and 85% respectively. In addition, it is rational to carry out studies to evaluate the functional parameters of serum albumin as a predictor of HCC in patients with chronic liver diseases.

Figure:



The receiver operating characteristic (ROC) curve of evaluated parameters in HCC diagnosis.

P07-06 Salvage AALDLT for HCC beyond all criteria yield equivilant results to Milan criteria in high prevalence young HCC patients country

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Background and aims: HEPATOCELLULAR CARCINOMA (HCC) is the second leading cause of cancer mortality worldwide. Living donor liver transplantation (LDLT) for HCC patients has emerged as a rewarding therapy for a cure and a successful alternative where a Deceased donor liver transplantation (DDLT) program is lacking. Therefore, trials for careful expansion to Milan criteria have been adopted

Aim of study is : To evaluate the impact of expanding criteria beyond Milan on tumor recurrence and patient survival that will help identify the best selection criteria for HCC transplanted patients

Method: The patient cohorts derived from Ain Shams Center for Organ Transplantation database between January 2004 and December 2015. This study excluded 15 patients,10 patients with incomplete follow-up, 2 patients missing essential data for analysis 3 patients pathology revealed no HCC.

241 patients were available for analysis; all pre-transplant, operative and post-transplant data were collected. All of participants were histologically confirmed by postoperative pathological examination.

Patients were divided according to the pre-transplant radiological findings of HCC nodules number and sizes into 3 groups:

Group I: comprised 175 patients who fall within the Milan criteria.

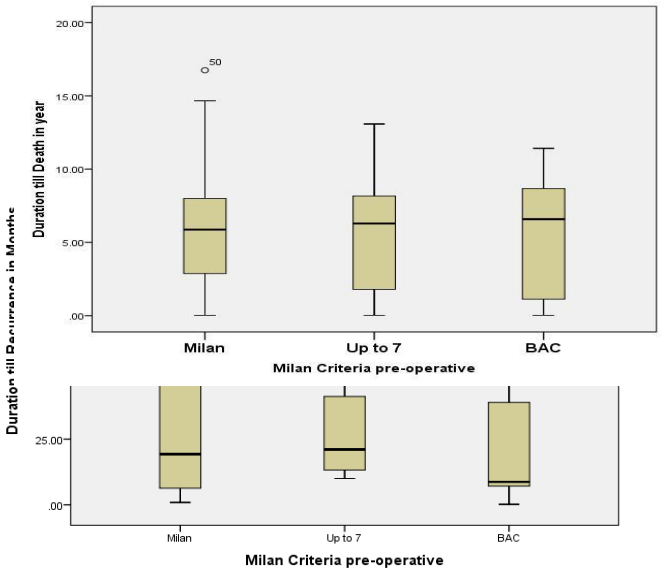
Group II: included 36 patients who fall within up-to-7 criteria (the sum of the number of the tumors and diameter of the largest tumor not exceeding 7cm)

Group III: included 30 patients beyond up-to-7 criteria and will be termed beyond all criteria (BAC).

Results: We followed up 241 patients transplanted for HCC their ages range from 28-67 with mean age 52.8.With 72.6%(N=175) fulfilled the Milan criteria, and 24.8%(N=60) provided the expansion criteria with16.8%(N=30) fulfilled the up to seven criteria and 14%(N=30) beyond all criteria. At the end of follow-up, 22 (12.5%) patients from the within Milian group and 6 (16.6%) patients from the up to seven group and 6 (20%) patients from beyond all the criteria experienced tumor recurrence, with no statistically significant difference between the 3 groups (P value=0.517) a Median time till recurrence was 19 months in Group I, 21 months in the up to seven and 8 months in the beyond all the criteria group. Recurrence of HCC was mainly extra-hepatic, there was statistically significant correlation between Microvascular invasion and undifferentiated tumour with HCC recurrence and interestingly preoperative AFP level is correlated with post transplant recurrence with area under the curve 0.661.

Conclusion: HCC Patients with expanded criteria seem to offer similar result for those within the Milan criteria in terms of recurrence and survival and LDLT is justified to expand the recipient pool.

Figure:
Median survival time in years in the 3 categories of HCC patients



Median TTR (time to recurrence non-significant P=**0.562 NS**

P07-07 Impact of corticosteroid therapy on the outcomes of hepatocellular carcinoma treated with immune checkpoint inhibitor therapy

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Background and aims: The impact of corticosteroid treatment (CT) on the efficacy of immune checkpoint inhibitors (ICI) in hepatocellular carcinoma (HCC) is undefined. We evaluated whether CT administered at baseline (bCT) or concurrently to ICI (cCT) influences clinical outcomes of HCC patients treated with ICI.

Method: This retrospective, multi-center observational study was conducted across 9 tertiary academic referral centers collected 341 HCC patients who received ICI across 3 continents between January 1, 2016 and April 1, 2019. Outcome measures included overall (OS) and progression-free survival (PFS) calculated from time of ICI commencement and overall response rates (ORR) defined by Response Evaluation Criteria in Solid Tumors (v1.1) on 6-8 weekly periodic restaging

Results: Of 331 eligible patients, 254 (76%) had BCLC-C stage HCC and received mostly PD(L)-1 ICI monotherapy (n=250, 85%). Median OS was 12.1 months (95%CI 9.2-15.0 months) and median PFS was 8.1 months (95%CI 6.3-10 months). In total 81 patients (24%) received $\geq$10 mg prednisone equivalent daily either as bCT (n=15, 4%) or cCT (n=66, 20%). Indications for CT included procedure/prophylaxis (n=37, 45%), management of irAE (n=31, 37%), cancer-related symptoms (n=5, 2%) or comorbidities (n=8, 3%). Neither overall CT, bCT nor cCT predicted for worse OS, PFS nor ORR in uni- and multi-variable analyses (p>0.05). CT for cancer-related indications predicted for shorter PFS (2.4 vs. 11.3 months, p=0.01), OS (4.5 vs. 12.8 months, p=0.05) and reduced ORR (p=0.03) compared to cancer-unrelated indications.

Conclusion: This is the first study to demonstrate that neither bCT nor cCT appear to influence response and OS following ICI in HCC. Worse survival and ORR in CT recipients for cancer-related indications appears driven by the poor prognosis associated with symptomatic HCC.

P07-08YI Possible hepatoprotective effect of chlorogenic acid and protocatechuic acid combinational approach against diethylnitrosamine induced hepatocarcinogenesis in rodents as a multitargeted ligand

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Background and aims: Hepatocellular carcinoma (HCC) has been reported as one of the most common lethal cancer. Due to restricted treatment choices, inevitable side effects and severe prognosis of hepatic cancer, there is a strict need of nature based preventive measure. Chlorogenic acid (CA) and protocatechuic acid (PA) are natural polyphenolic compounds that possess antitumor activity and cause apoptosis as well as autophagy in different types of malignancies like hepatocellular carcinoma. The present investigation was designed to evaluate the synergistic anticancer potential of IL-6combination in male Sprague Dawley rats along with its biochemical assays.
Method: HCC was induced by administering the diethylnitrosamine (DEN, 200mg/kg), and the test drug treatment (CA+PA) was consecutively provided by oral gavages at two different dose levels 10 and 20 mg/kg for 16 weeks. On last day of study, various antiproliferative parameters were determined including haematological profile, serum biomarkers, antioxidants enzymes, membrane bound enzymes and inflammatory cytokinase levels for each group. Histopathological changes were also assessed to confirm the protective effect via liver architecture.
Results: Results demonstrated that CA+PA administered group significantly downregulated ($P<0.01$) the serum marker hepatic and non-hepatic enzymes and proinflammatory markers such as tumor necrosis factor-α (TNF-α), interlukin-6 (IL-6) and interlukin-1β (IL-1β) as compared to DEN alone group. A noteworthy and dose dependant elevation was also observed in the levels of enzymatic and non-enzymatic antioxidants status. Histopathological slide of different groups also supported the protective effect of CA+ PA against HCC in a dose dependant manner through attaining the normal hepatocellular structure.
Conclusion: Our data recommended that combination approach of CA+PA promisingly inhibited the DEN induced damaging effects on liver via regulating the antioxidant defense system as well as proinflammatory cytokines and it may be utilized as a better option to modernize the clinical results against HCC.

P07-09YI Patient-led functional genomics identifies novel drivers of intrahepatic cholangiocarcinoma

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Background and aims: Intrahepatic cholangiocarcinoma (CCA) is characterised by genetic heterogeneity which, along with small cohort sizes, complicates complete discovery of low-frequency drivers active in this cancer. For the subset of patients that do not harbor known drivers, this is particularly problematic as it precludes genetically targeted therapy. The identification rare driver genes active in only a minority of patients will help us better understand the basic biology of CCA as well as inform therapeutic strategies for this group.

Method: Aggregation and re-analysis of published exome-seq data with frequency-independent driver prediction tools identifies a set of candidate drivers. In vivo screening of these candidates in the adult mouse liver using CRISPR/Cas9 screening libraries was carried out. Resultant tumours were characterized with exome-seq, RNA-seq and histology to determine CRISPR/Cas9-induced mutations and phenotypic changes.

Results: Computational driver prediction identified a set of 98 predicted drivers, mostly found in <5% of patients. In vivo screening of this set in the adult mouse liver produced tumours within 10 weeks. Genome and transcriptome analysis of resulting tumours defined a set of rare drivers that are enriched for canonical roles in developmental processes both in the liver and other organ systems. In vivo validation of individual hits, both alone and in combination with more common, known drivers, showed that their loss results in aggressive, invasive, and poorly differentiated CCA. Specifically, interaction of these drivers with the more common KRAS and NRAS oncogenes modulates phenotype and increases tumour onset and mortality.

Conclusion: Combining bioinformatic and experimental screening further elucidates the genetics and biology of CCA, particularly in the subset of patients without known driver genes. Overall, this study identifies novel drivers of CCA and additionally identifies potential therapeutic targets when mutated in combination with the more common RAS drivers, for which direct targeting is a challenge across cancer types.

P07-10YI NK-cell dysfunction in Hepatocellular Carcinoma: modulatory approches for functional restoration

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Background and aims: Several immune mechanisms contribute to an immunosuppressive tumour immune milieu: regulatory immune cells, checkpoint receptors, low nutrients and hypoxia that can affect immune cells metabolism and function. Metabolic profiles rather than phenotypic characteristics seem to define the functional role of NK cells and their influence in a correct anti-tumour effect. NK-cell response is functionally impaired in Hepatocellular Carcinoma (HCC), however little is still known about NK-cell metabolism in this tumour.

Method: Liver and tumour infiltrating lymphomononuclear cells from 10 patients were derived from surgical specimens and NK-cells were purified by flow activated cell sorting in order to perform gene expression profiling. NK-cell phenotype, glucose uptake and mitochondrial polarization were defined by multi-parametric flow cytometry. Modulatory compounds were identified on the basis of gene expression profiling and employed to restore tumour infiltrating NK-cells metabolism and cytotoxic functions.

Results: Genome-wide expression profiling showed enrichment of upregulated genes belonging to metabolic pathways in HCC-infiltrating NK cells: glycolysis and oxidative phosphorylation, cell cycle, DNA damage and p38-related pathway. Higher level of phosphorylated-p38 protein was confirmed in tumour infiltrating NK cells while phenotypic characterization showed enrichment of CD49a positive and CD27CD11b double negative NK-cells that has been associated with regulatory and dysfunctional NK-cells. Functional and metabolic assessment showed defective degranulation capacity, reduced autophagy and glucose consumption. Targeting MAPK pathway by two different p38-inhibitors could restore NK-cell functions.

Conclusion: We identified an impairment of energy metabolism of HCC-infiltrating NK-cells associated with functional defects that can be rescued modulating MAPK pathway. These results provide the basis to develop new strategies to potentiate NK-cell response in HCC.

P07-11YI Safety comparison between drug-eluting beads and conventional transarterial chemoembolization for hepatocellular carcinoma: A multicentre study

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Background and aims: The selection between drug-eluting beads transarterial chemoembolization (DEB-TACE) and conventional transarterial chemoembolization (cTACE) for the treatment of hepatocellular carcinoma (HCC) is still controversial. Compared to cTACE, limited data are available so far concerning circulation toxicity and liver-specific toxicity in DEB-TACE.

To evaluate the safety on the treatment of HCC with DEB-TACE in comparison with cTACE.

Method: In this multicentre, retrospective study, 1002 treatment-naïve patients undergoing DEB-TACE or cTACE between May 2016 and November 2018 were enrolled. The primary endpoint was treatment safety as assessed by treatment related complications and deaths. Liver/biliary injuries was assessed by imaging including dilated bile duct, portal vein narrowing, portal vein thrombosis, liver infract, and intrahepatic biloma. Liver toxicity assessment included serum hepatic function variables and hepatic related complications. Post-embolization syndrome was defined by the post-procedural occurrence of fever, abdominal pain, vomiting, fatigue, abdominal distension, nausea, hiccups and constipation. Circulation toxicity included bone marrow suppression and granulopenia. Adverse events (AEs) were assessed during the perioperative period and the follow-up period according to the NCI Common Terminology Criteria for Adverse events (CTCAE) version 5.0.

Results: The incidence rates of bile duct dilation, portal vein narrowing and liver failure in the DEB-TACE group were 15.48%, 4.57%, 2.28%, respectively, which were significantly higher than those in the cTACE group ($P < 0.001$, $P = 0.006$, and $P = 0.026$, respectively). There was no significant difference in the incidence of post-embolization syndrome and circulation toxicity except abdominal pain, which was 1.5 and 3 times more frequent in grade 2 and grade 3, respectively, in the DEB-TACE group compared to the cTACE group ($P < 0.001$).

Conclusion: DEB-TACE was associated with more frequent liver/biliary injuries and severe abdominal pain compared to cTACE in patients with HCC. Given this finding, no significant superiority concerning safety can be made for DEB-TACE compared with cTACE for the treatment of HCC. More attention should be payed to the safety from DEB-TACE.

Figure:

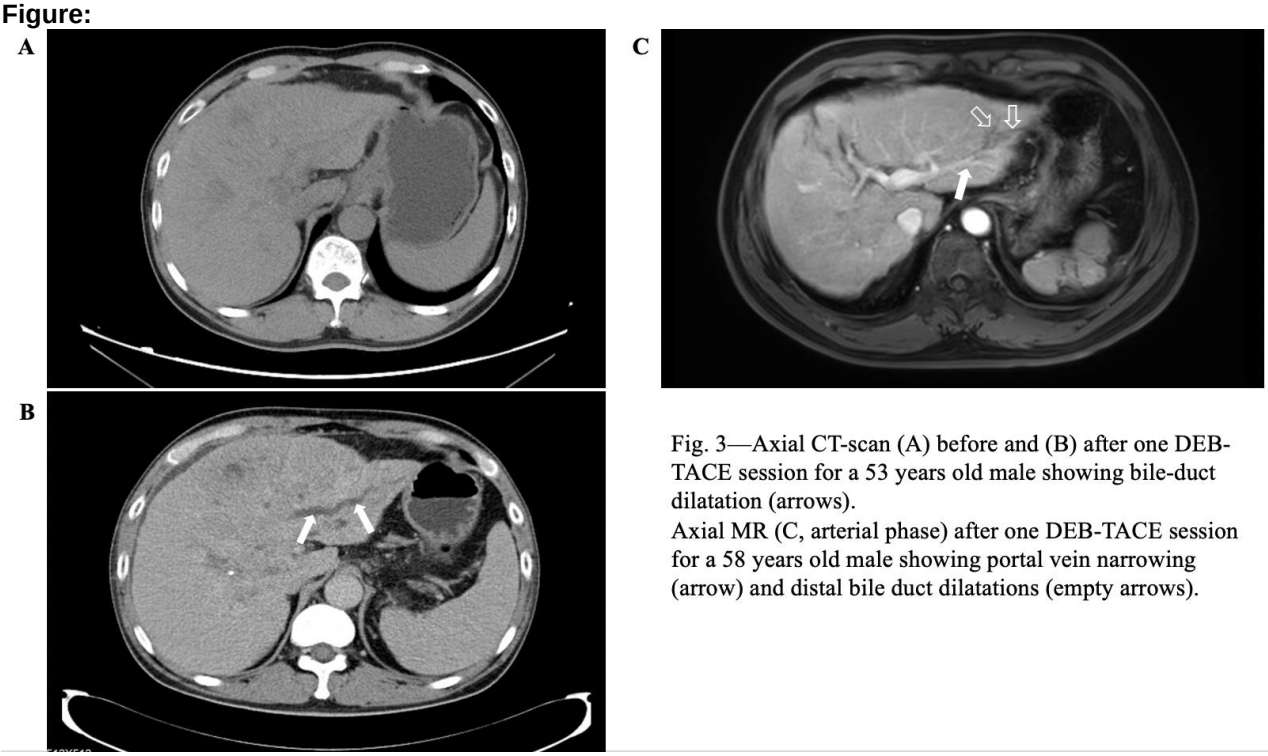


Fig. 3—Axial CT-scan (A) before and (B) after one DEB-TACE session for a 53 years old male showing bile-duct dilatation (arrows).
Axial MR (C, arterial phase) after one DEB-TACE session for a 58 years old male showing portal vein narrowing (arrow) and distal bile duct dilatations (empty arrows).

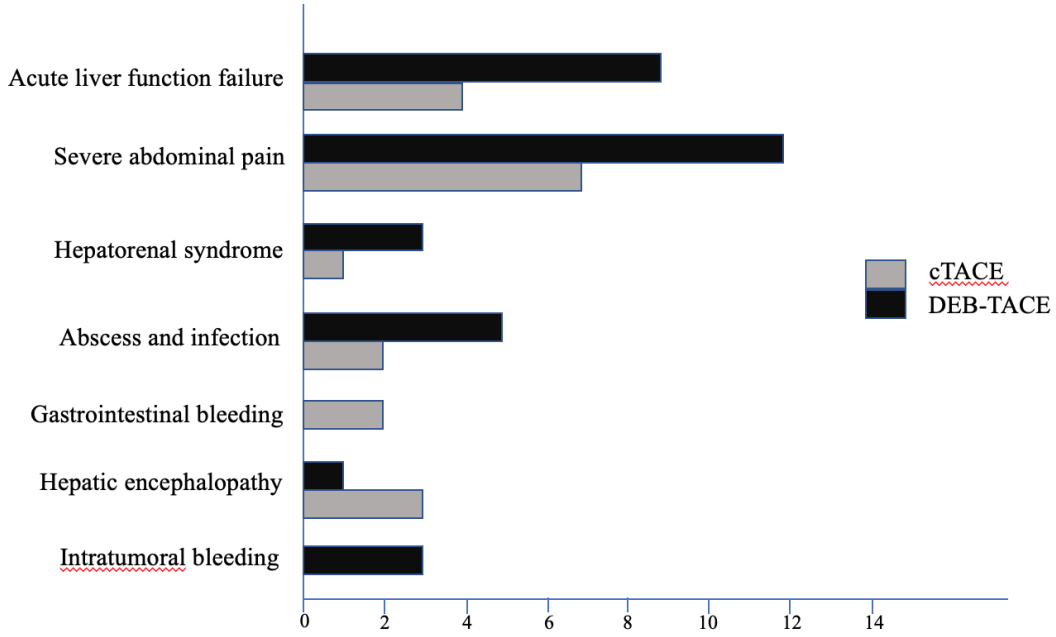


Fig. 6—Graph shows frequency of serious adverse events (SAEs) in both groups: drug-eluting beads transarterial chemoembolization (*black*) and conventional transarterial chemoembolization (*gray*).

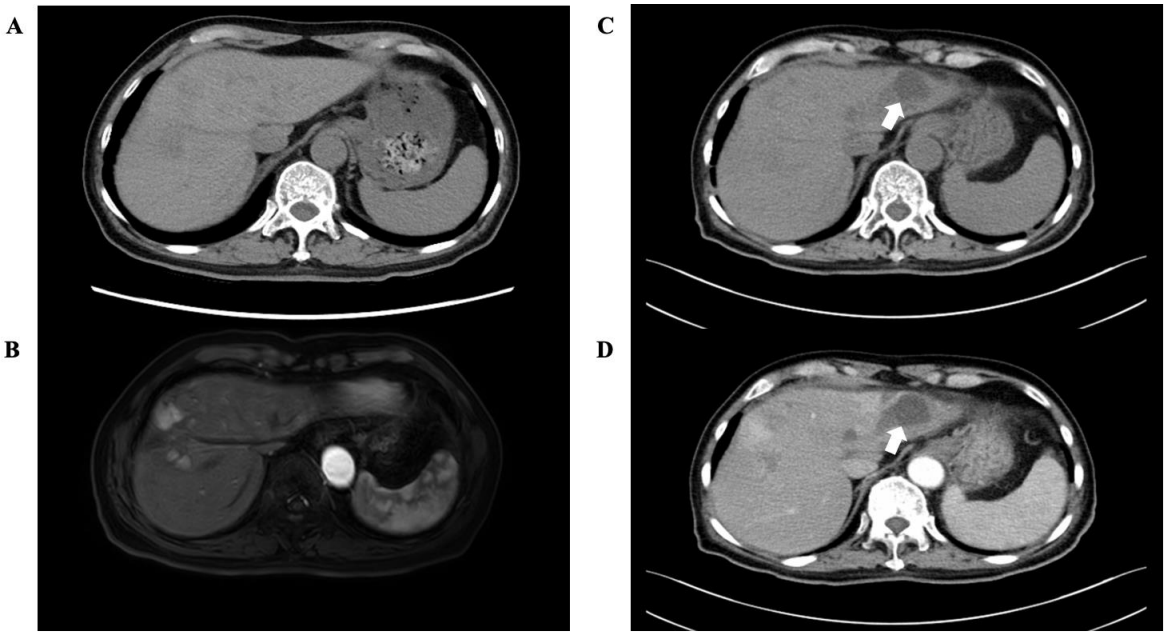


Fig.4—Axial CT-scan (A) and MR (B, arterial phase) before and after (CT-scan (C) and arterial phase (D)) one DEB-TACE session for a 65 years old male with HCC showing 4 cm biloma of the left liver lobe (arrow) without enhancement after injection of iodine contrast material.

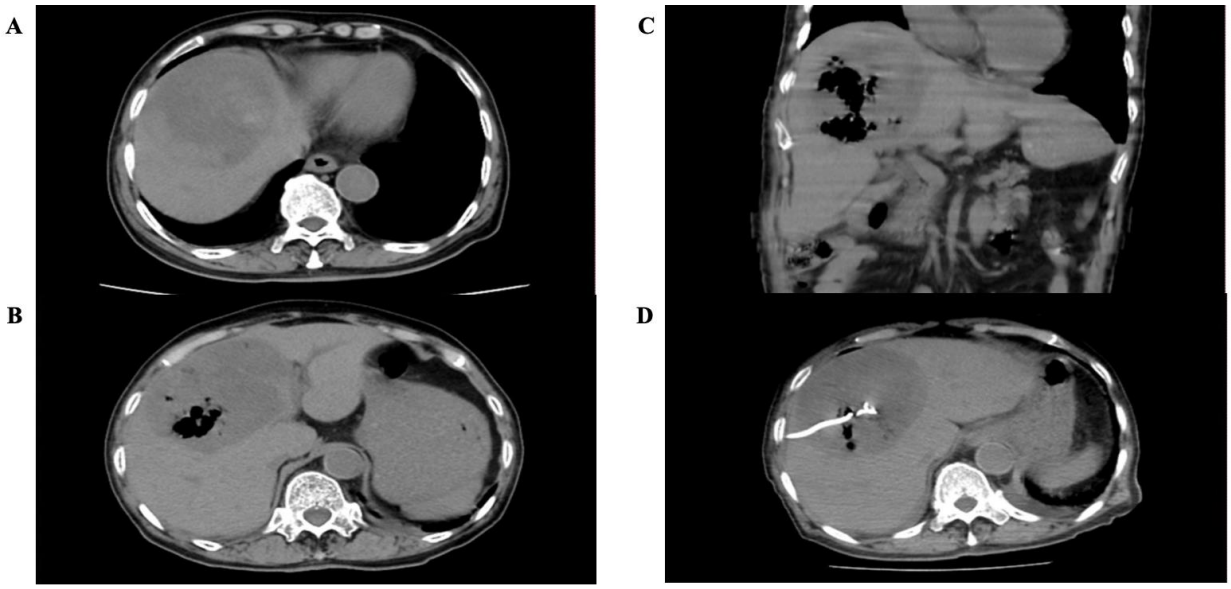
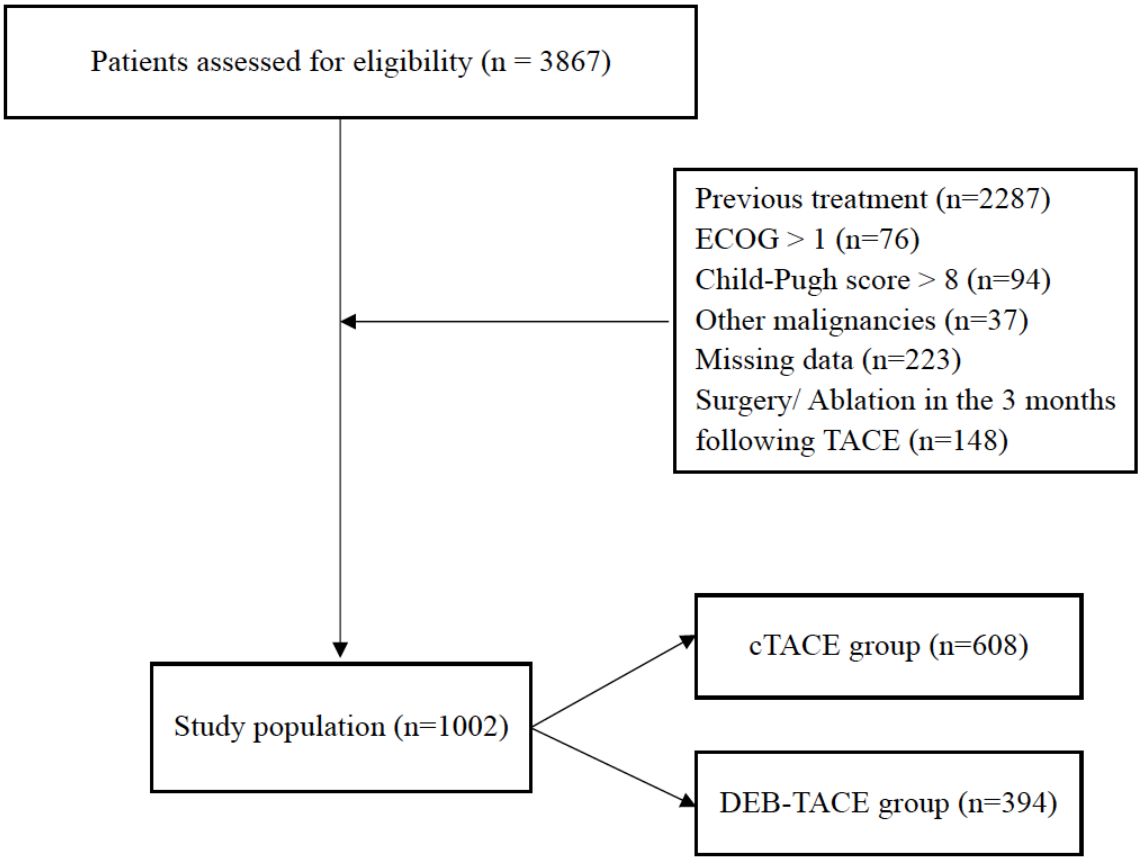


Fig.5—Axial CT-scan (A) before second DEB-TACE session for a 88 years old male with HCC; axial CT-scan (B) shows a small amount of gas in the tumor in 3 days after the procedure; the patient developed chills and fever in 10 days after the procedure and axial CT-scan (C) shows the progression of liver abscess; the patient was performed with puncture drainage (D), and died in 3 weeks after the procedure due to severe infection.



P07-12YI The combination of EpCAM-positive circulating tumor cells and serum AFP/AFP-L3/DCP predicts outcome after curative resection of hepatocellular carcinoma

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Background and aims: Early hepatocellular carcinoma (HCC) has a limited prognosis due to recurrence rates of more than 50% after liver resection. As reported earlier, EpCAM-positive circulating tumor cells (CTC) have a high predictive value for presence of micro-metastases and early HCC recurrence after resection, however, sensitivity remains low (22%) (von Felden, Schulze et al., Oncotarget 2017). The serum biomarker-triplet alpha-fetoprotein (AFP), lectin-reactive AFP (AFP-L3), and des-gamma-carboxy prothrombin (DCP) are well-known diagnostic and prognostic markers for HCC. The objective of this study was to evaluate a composite panel of CTC and the biomarker-triplet to identify patients with high risk of early recurrence after liver resection.

Method: We prospectively enrolled patients undergoing curative intended resection for HCC at a single institution between 2011 and 2015. Blood specimens were obtained prior to resection and processed with the CellSearchTM system, detecting EpCAM-positive CTC, and serum levels of AFP, AFP-L3, and DCP were measured with the µTASWako™ system. The primary endpoints were early recurrence within two years after resection, recurrence-free survival (RFS), and overall survival (OS).

Results: A total of 66 patients were prospectively enrolled (86% male, age 66 years, 84% early disease with one or two nodules). A positive test was defined as detection of CTC or any serum marker AFP, AFP-L3, or DCP above the local thresholds, and significantly associated with shorter RFS (7 vs. 20 month, p=0.011) and OS (21 vs. not reached, p=0.036). Multivariate regression analysis for early recurrence including the composite biomarkers, vascular invasion (V0 vs. V1/2), and status of resection margins (R0 vs. R1/2), revealed hazard ratios of 2.12 (95% confidence interval [95%-CI] 0.9-5.0, p=0.084), 2.01 (95%-CI 0.92-4.4, p=0.081), and 0.95 (95%-CI 0.3-3.0, p=0.925), respectively. The sensitivity for the composite biomarkers to detect patients who will develop an early recurrence after surgery was 73%.

Conclusion: An easy to use blood-based composite test for detection of EpCAM-positive CTC and biomarkers AFP, AFP-L3, and DCP improved the sensitivity for the prediction of early recurrence, likely due to the presence of micro-metastasis at time of resection, and was able to discriminate outcome following curative-intended liver resection. Thus, the combination potentially identifies patients with more aggressive tumors who might benefit from adjuvant treatment.

P07-13YI Long-term prospective study of development of hepatocellular carcinoma in compensated cirrhosis

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Background and aims: Hepatocellular carcinoma (HCC) is the 5th most frequent incident solid tumor in males and the 2nd for mortality. Main risk factors for HCC development have been mostly identified in retrospective cohorts. With the aim of identifying clinical and biologic risk factors for HCC development, we started in 2013 a prospective study in patients with liver cirrhosis undergoing hepatic venous pressure gradient (HVPG) measurement (ClinicalTrials.gov Identifier: NCT03083002).

Method: 445 consecutive patients with liver cirrhosis (66.5% CP-A, 23.2% CP-B, 10.3% CP-C) undergoing HVPG measurement and transjugular liver biopsy at the Gastroenterology Unit, Modena, were enrolled in this prospective study starting July 2013. Patients were followed up every 6 months with US and blood tests. Those developing HCC in the first 6 months of follow-up were excluded. Data regarding etiology, portal vein, portal vein thrombosis, HVPG, presence and grading of esophageal varices, Child-Pugh and MELD Score, time of development of HCC were collected. Incident cases of HCC were biopsied for pathological and transcriptomic characterization. Independent risk factors for HCC were evaluated by Cox regression analysis.

Results: Median follow up was 40 months. 61 patients died during follow up, 34 developed HCC (incidence 4-5% per year). Preliminary results of analysis of hepatic and circulating biomarkers of angiogenesis, portal hypertension and fibrosis as predictive factors for HCC development indicate marked activation of angiogenesis as related with HCC risk. At univariate analysis HVPG>15 (but not HVPG>10 or >20 mmHg), F2/F3 esophageal varices, viral vs. non-viral etiology, and albumin were statistically associated with HCC development (HVPG/F2-F3 varices collinear). In multivariate model with HVPG>15, none of these factors was significantly associated with HCC development while in model with F2/F3 esophageal varices, presence of the latter was independently linked with HCC development (HR 2.258, 95% CI 1.135-4.494). Albumin only had borderline significance (.586, CI% .337-1.018).

Conclusion: In this prospective cohort of patients with compensated liver cirrhosis, the only risk factor for HCC development was the presence of F2/F3 varices. Neither HVPG>10 not >15 or >20 was independently associated with HCC development. Previous data indicating HVPG>10 as a significant risk factor for HCC were likely influenced by the cohort studied, which was free of varices at enrollment. In a cohort of compensated patients with liver cirrhosis not selected for absence of varices, more severe portal hypertension as indicated by F2/F3 varices was the only independent risk factor for HCC development.

P07-14 Characteristics of hepatocellular carcinoma post direct antiviral treatment of chronic hepatitis C patients: Comparative study with non-direct-acting antivirals-treated patients

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Background and aims: HCC is the first related cancer in the total population in Egypt. Introducing of the new direct acting antiviral (DAA) has revolutionized the management of HCV worldwide, with high rates of sustained viral response reached more than 95% in most strains. More than 2 million egyption HCV infected patients have been treated by DAA till October 2018. HCC post direct acting seems to be different in many patients from that without DAA treatment in certain aspects as noted in recent studies. Our aim is to evaluate clinical, laboratory, radiological, and histological characteristics and biological behaviour of HCC patients post DAA
Method: Case control study of HCC patients presented to our multidisciplinary HCC committee in NLI(National Liver Institute) Shbin Elkom, Menofia University from January 2015 till October 2019.Patients was divided into group I HCC patients post DAA 2036 patients and group II HCC patients 60338 not treated by DAA. Demographic, clinical, performance status, laboratory, radiological and histopathological examination of the resected lesions performed. Our study is a comparative study trying to evaluate the characterization and the biological behavior of the HCC lesions in post DAA treated patients and in HCC patients without DAA treated
Results: In group I AFP, portal vein invasion, local lymph node metastasis and distant metastasis is significantly present in higher rates than group II. MVI and infiltrative lesions is significantly present in higher rates in group I than group II. HCC patients post DAA treatment in group I seems to be more aggressive in behavior than HCC patients not treated by DAA treatment.
Conclusion: Regarding the aggressive behavior of the HCC lesions post DAA strict surveillance should be done in treated cirrhotic patients by 3 months abdominal ultrasound and AFP in developing countries and triphasic CT abdomen in developed countries according to the socioeconomic status.
Figure: Triphasic CT scan of the liver showing a large right lobe HCC exophytic focal lesion(segment VI) in a 50years old HCV positive male patient received DAA therapy .The arterial phase showing a large exophytic peripherally enhanced lobulated surface lesion with intratumoral arteries, multinodularity and incomplete lesion capsule right, wasout is seen in portal and delayed phases middle and left
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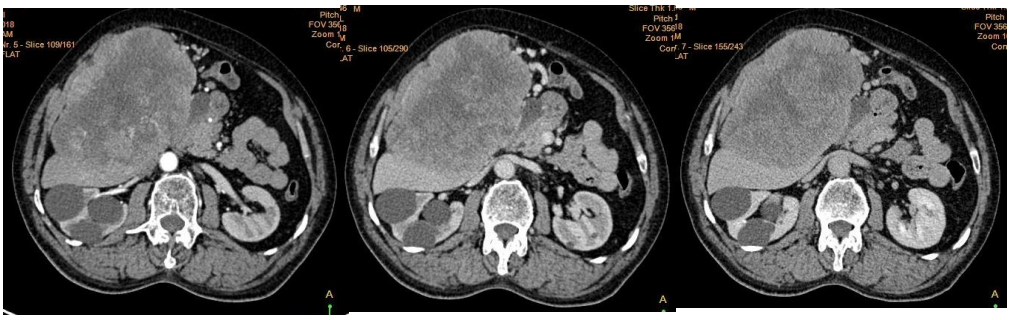

P07-15 Common polymorphisms in the interleukin-1 beta gene are associated with HCV-related HCC in Caucasian patients

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Background and aims: Interleukin-1beta (IL-1b) is a proinflammatory cytokine which plays a pivotal role in hepatocarcinogenesis. Previously, we reported an association of common polymorphisms in the IL-1b gene with hepatitis B virus-related hepatocellular carcinoma (HCC) in Caucasians. In the present study, we assessed the frequency of these polymorphisms in hepatitis C virus (HCV)-related HCCs.

Method: In this retrospective study, 101 patients with chronic HCV infection and HCC and 124 matched HCV controls without HCC were enrolled. 74 % of patients had genotype 1 (GT1), 4 % GT2, 17 % GT3, 5 % GT4). The mean age was 55 ± 12 years and 56 % were male. Liver cirrhosis was diagnosed in 96 (43 %) patients. Host genomic DNA was extracted from peripheral blood samples. Genotyping of IL-1b polymorphisms rs1143623, rs1146327 and rs16944 was performed.

Results: The genotype distribution of IL-1b rs1143623, rs1146327 and rs16944 differed between patients with and without HCC. Thus, frequencies of rs1143623 CC, rs1146327 TT and rs16944 CC were significantly higher in patients with HCC than in patients without HCC (rs1143623 CC: 64 % vs. 51 % p = 0.044, rs1146327 TT: 54 % vs. 39 % p = 0.032, and rs16944 CC: 54 % vs. 40 % p = 0.031). The three genotypes were associated with a higher likelihood of HCC presence with odds ratios (OR) of 1.75 for rs1143623 CC (p = 0.042), 1.82 for rs1146327 TT (p = 0.028) and 1.83 for rs16944 CC (p = 0.026). In multivariate analysis, the rs16944 TT remained associated with HCC (OR = 1.84, p = 0.027). In haplotype analysis, the SNPs rs1146323 and rs1143627 were in complete linkage. The haplotype including both variants rs1143623 TT and rs16944 CC was a risk factor for HCC development (OR = 1.66 p = 0.017).

Conclusion: Polymorphisms in the IL-1b gene are associated with HCC development in HCV infections. Since hepatocarcinogenesis is affected by inflammatory processes, we suggest that variations in genes coding for cytokines might be considered as HCC risk factors regardless of the etiology of the underlying liver disease. A validation of our findings in cohorts with HCC related to different causes of liver disease is required.

P07-16 Incidence of indeterminate hepatic nodules in the preoperative magnetic resonance imaging in patients with colorectal cancer, their diagnosis, management and clinical outcome

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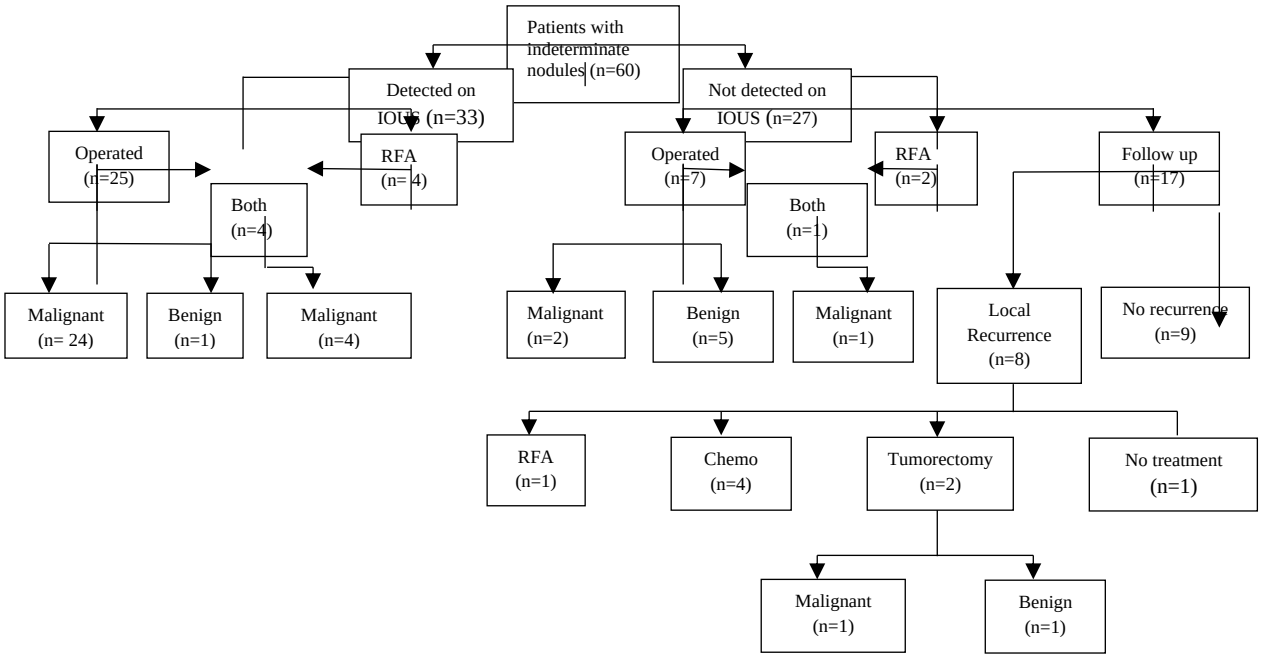
Background and aims: With a shift in the management of colorectal liver metastasis (CRLM) there is increasing need to accurately diagnose metastatic lesions and manage them appropriately. In spite of CT scan being the investigation of choice, there in growing evidence indicating MRI with hepatocyte specific contrast may be superior, especially for diagnosis of small indeterminate nodules on CT. However, there are still some nodules which remain indeterminate and new nodules which are identified on MRI. The management of these nodules is uncertain. Our objective is to study the natural course of these nodules and evaluate treatment strategies for the same.
Method: Out of the 473 patients who underwent surgery for CRLM, 389 were analysed with Gadoxetic acid MRI. Indeterminate nodules were found in 60 patients (15.42%). These 60 patients were then evaluated with an intraoperative ultrasound (IOUS). Those identified on IOUS were operated or RF ablated, the remaining were followed up.

Results: The incidence of patients with indeterminate nodules on MRI was 15.42% with most being male (71.67%). The median age of patients was 61 years. Synchronous lesions were found in 66.67% (40) while 71.67% (43) had solitary indeterminate nodules. IOUS was found to have a sensitivity and specificity of 73.68% and 93.75% respectively for indeterminate nodules, with a positive predictive value of 96.6%. Patients whose nodules were ablated were excluded. Majority of the patients followed up had benign nodules 58.82% (10/17). While comparing the benign and malignant nodules in the followed up group, pLNR levels(ratio of positive lymph nodes to total number of lymph nodes resected in the primary) were higher in the malignant group and this was statistically significant (p=0.006). There was no significant relationship with either parameter individually.

Conclusion: IOUS should be used as an adjunct to MRI in patients with indeterminate nodules owing to its high positive predictive value. pLNR could be used as a tool to decide which patients can be conserved at least in the metachronous setting. There is a need for prospective studies with larger number of patients to identify additional clinico-pathological criteria so as to predict the malignant potential of these lesions.

Figure: : Natural Course of Indeterminate Nodules



P07-17YI Risk of developing hepatocellular carcinoma in patients with cirrhosis of viral etiology evaluated by the combination of alpha-fetoprotein, protein induced by vitamin K absence or antagonist-II and glypican-3

Gian Paolo Caviglia[1], Maria Lorena Abate[1], michela ciruolo[2], Patrizia Carucci[2], Antonella Olivero[1], Emanuela Rolle[2], Chiara Rosso[1], Ramy Younes[1], Alessia Ciancio[1], Antonina Smedile[1], Giorgio Maria Saracco[1 2], Elisabetta Bugianesi[1 2], Silvia Gaia[2]
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Background and aims: International guidelines recommend the use of ultrasound as surveillance tool for hepatocellular carcinoma (HCC) in patients with cirrhosis, while the role of serum biomarkers is still a matter of debate. We investigated serum alpha-fetoprotein (AFP), protein induced by vitamin K absence or antagonist II (PIVKA-II) and glypican-3 (GPC-3) diagnostic accuracy for HCC detection and prediction in patients with cirrhosis of viral etiology under surveillance.

Method: A total of 244 patients with either cirrhosis (n = 120, 74M/47F, median age 57 [33 - 82] years, 91 HCV and 30 HBV) or HCC (n = 124, 103M/21F, median age 66 [31 - 89] years, 94 HCV and 30 HBV; 76.7% early HCC) were retrospectively enrolled. We also analyzed data from a group of patients with HCC (n = 27, 21M/6F, median age 67 [53 - 78], 14 HCV and 13 HBV) with available serum samples collected at 9 and 18 months before HCC diagnosis. AFP and PIVKA-II serum values were measured by chemiluminescent immunoassays on Lumipulse® G600 System (Fujirebio Inc, Tokyo, Japan) while GPC-3 by enzyme immunoassay (Fujirebio Diagnostic AB, Gothenburg, Sweden).

Results: AFP, PIVKA-II and GPC-3 serum levels were lower in patients with cirrhosis than in those with HCC (AFP: 7.4 [95%CI 6.0 - 9.0] vs 20.2 [95%CI 14.7 - 31.5] ng/mL, $p < 0.001$; PIVKA-II: 43 [95%CI 41 - 47] vs 126 [95%CI 96 - 176] mAU/mL, $p < 0.001$; GPC-3: 0.07 [95%CI 0.06 - 0.10] vs 0.14 [95%CI 0.11 - 0.18] ng/mL, $p < 0.001$). The higher performance for HCC detection was observed for PIVKA-II (area under the curve [AUC] = 0.804), followed by AFP (AUC = 0.734) and GPC-3 (AUC = 0.626); the combination of AFP + PIVKA-II + GPC-3 furtherly improved the diagnostic accuracy to AUC = 0.833, with sensitivity of 69% and specificity of 88% at a cut-off of 0.51 (Youden Index). Serum AFP, PIVKA-II and GPC-3 values distinctly increased from 18 to 9 months prior HCC detection and additionally to HCC diagnosis (Friedman test, $p < 0.05$). The combination of AFP + PIVKA-II + GPC-3 enabled to discriminate between patients who developed HCC (n = 27) from those who did not (n = 120) as early as 18 months before tumor diagnosis (Log-rank test, $p = 0.002$) and to predict HCC development (hazard ratio = 3.13, 95%CI 1.47 - 6.65, $p = 0.003$).

Conclusion: The combination of AFP + PIVKA-II + GPC-3 showed a good accuracy for HCC detection and may allow the identification of cirrhotic patients under surveillance at higher risk of HCC development.

P07-18YI Composite targeting of nuclear receptors protects mice from NAFLD progression towards HCC

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Background and aims: The molecular mechanisms governing non-alcoholic fatty liver disease (NAFLD) triggering and progression remain incompletely understood. We recently showed that concomitant miR-21 ablation and farnesoid X receptor (FXR) activation prevents NAFLD development in mice. Here, we aimed to elucidate whether INT-767, a dual FXR and G protein-coupled receptor 5 (TGR5) agonist, alone or in combination with miR-21 silencing, could synergize in preventing non-alcoholic steatohepatitis (NASH) progression towards cirrhosis and hepatocellular carcinoma (HCC).
Method: C57BL/6N mice were fed a high-fat choline-deficient (HFCD) diet for 24 weeks, supplemented with or without INT-767, and/or injected with antagomiR-21 or a scrambled antagomir (control). Liver and serum samples were collected and processed for histological and molecular assessment. Expression of genes and proteins involved in NAFLD signalling circuits, as well as those lying downstream of activation of nuclear receptors, were investigated through qRT-PCR and immunoblotting.
Results: HFCD diet-fed mice developed NASH with significant fibrosis and, notably, pre-neoplastic nodules. In turn, INT-767 and antagomiR-21, alone or in combination, prevented fibrosis instalment and development of pre-neoplastic nodules. At the molecular level, HFCD diet-induced upregulation of liver pro-inflammatory genes (IL-1β, TNF-α, IL6, IL8, NLRP3, TLR4) and proteins (NF-κB, *IκB*-α, p-c-Jun, p-JNK), as well as pro-fibrogenic markers (collagen1α1, α-SMA, TGF-β), were significantly reverted by either INT-767 or antagomiR-21 alone, and even more so, by combined treatments. Further, HFCD diet-induced modulation of apoptosis- and necroptosis-related proteins (Caspase-1, -2, -3, RIP1, RIP3), as well as mitochondrial proteins (DRP-1, FIS-1, MFN2, OPA-1, NRF1, NRF2, TFAM) was similarly prevented upon both treatments. Finally, HFCD diet-induced downregulation of lipid metabolism genes/proteins was rescued by either INT-767 or antagomiR-21 alone, as well as both combined.
Conclusion: In conclusion, either INT-767 or antagomiR-21 alone prevent NAFLD progression, with combined targeting more effectively preventing progression towards pre-neoplastic lesions and, ultimately, HCC, thus embodying a putative therapeutic strategy.
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P07-19YI Variable expression of venous clot coordinator high mobility group box-1 in patients with hepatocellular carcinoma with or without portal vein thrombosis

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Background and aims: High Motility Group Box 1 (HMGB1), whose expression is stimulated by hypoxia and carcinogens, orchestrates inflammation-induced venous thrombosis. We aimed to contribute to better understanding the pathophysiological role of HMGB1 in patients with hepatocellular carcinoma (HCC) complicated or not by portal vein thrombosis (PVT).

Method: We prospectively recruited N=100 patients with newly diagnosed HCC (as for 2018 EASL guidelines), categorized into two subgroups: group A (N=78, without PVT) and group B (N=22 with PVT). We analysed blood samples from each patient and from N=34 healthy subjects (group C). Serum (groups A-C) and plasma (group C) HMGB1 concentration was measured by the HMGB1 ELISA test (IBL International GmbH, Hamburg, Germany). Moreover, platelet expression of HMGB1 was analysed in patients with HCC and healthy subjects by western blot, comparing it to that in nuclear and cytoplasmic fractions obtained from a human hepatoma cell line (HuH-7).

Results: Median serum HMGB1 among HCC patients (N.=100) was 7.8 ng/mL (4.8-10.5) vs. 4.09 ng/mL (3.08-8.20) in group C (p= 0.006). In group A, however, median serum HMGB1 was 8.2 ng/mL (5.5-13.1), while it was 5.5 ng/mL (4.4-8.5) in group B (p=0.012); the difference between group B and group C was not significant (p=0.512). At multivariate analysis, among a set of variables including age, sex, Child-Pugh class, number of HCC nodes, platelet count, size of the largest HCC node and serum HMGB1 concentration, only the latter two variables were independent predictors of PVT (OR= 1.05, 95% CI 1.02-1.07, p= 0.001 and OR 0.75, 95% CI 0.63-0.91, p= 0.003, respectively). In Group C, the median plasma concentration of HMGB1 was 0.65 ng/mL (0.39-1.06), approximately 7-fold lower than serum concentration measured at the same time point (p< 0.001). In healthy subjects and HCC patients without PVT, platelet expression of HMGB1 was shown as a single band of molecular weight approximately 50 kDa (coherent with the band observed in the cytoplasmic fraction of HMGB1). In the platelets of HCC patients with PVT, two additional HMGB1 bands of approximately 45-75 kDa were present.

Conclusion: Most of serum HMGB1 originates from the process of coagulation induced in vitro, mostly reflecting how much of this protein can be mobilized from platelets. In the presence of PVT, HMGB1 undergoes significant quantitative and qualitative changes, possibly related to its association with other (inflammatory?) proteins.

P07-21 Advances in serum biomarkers for primary and secondary liver carcinomas

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Background and aims: Early diagnostics and tumor aggressivity estimation are crucial for an optimal surgery treatment of both primary liver carcinoma and liver metastases. However, both processes (primary and metastatic ones) may require individual and different approaches. Regarding the primary liver cancer it seems to be important to identify the risk group of patients to be involved in screening programs. Metastatic liver process will require the optimal follow-up proposal particularly in colorectal and breast cancer in order to make a good decision whether to perform the surgery resection instead of palliative therapy or chemotherapy. Serum biomarkers may help in early diagnostics of cancer, differential diagnosis between benign and malign processes, reduction of imaging examinations, estimation of prognosis and optimal proposal for therapy procedure, and optimal follow-up. However, „classical" tumor markers such as alpha-feto protein (AFP) have low sensitivity and specificity. Other molecules, related to liver processes such as fibrosis and cirrhosis could be potential candidate biomarkers. Results of several studies including our pilot study1 suggest that serum PIVKA II (protein induced by vitamin K absence-II) has better sensitivity than serum AFP. A combination of several biomarkers could further enhance the sensitivity. The aim of the study was to find an optimal combination of biomarkers for the diagnostics of HCC and liver metastases.
Method: We studied serum levels of PIVKA II, AFP and liver fibrosis markers (hyaluronic acid, procollagen PIIINP and TIMP1) together with tumor markers CA 19-9, CEA and TPS in the group of patients with HCC (n=24), in the group of patients with liver metastases (n=98), in the group of patients with benign liver disease (n=15) and in healthy controls. The immunoanalytical methods were used for biomarker assessments.
Results: Serum AFP and PIVKA II were significantly elevated in primary liver carcinoma group ($p < .0001$ both) but not in liver metastases group ($p = 0.7108$ and 0.0645, respectively). Cytokeratin TPS was significantly elevated in both groups ($p < .0001$ both). The highest sensitivity (at 95% specificity) was achieved for PIVKA II (AUROC 0.8531) followed by AFP (AUROC 0.7710). Very low sensitivity was found for CA 19-9.
Conclusion: Our research suggests that PIVKA II together with AFP are best markers for hepatocellular carcinoma. Moreover, serum CEA in combination with cytokeratin TPS are promising for the evaluation of liver metastases aggressivity. Liver fibrosis markers levels could help in gaining knowledge about the etiology and pathophysiology of the processes in liver.

PB01-01-YI Neoangiogenic transcriptomic signature identifies HCCs with worse response to treatment: Long-term results of a prospective study

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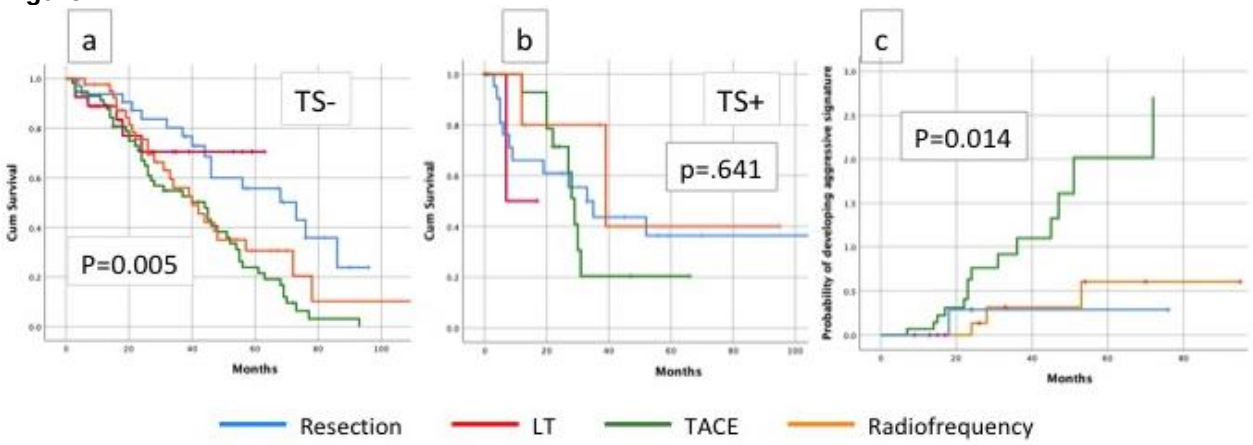
Background and aims: Hepatocellular carcinoma (HCC) is the 2nd solid tumor for mortality in males. Current therapeutic algorithms, e.g. BCLC, do not take into account molecular signatures for choosing treatment, evaluating response and survival. We prospectively evaluated outcome of treatments performed according to SOC in relation with the neoangiogenic transcriptomic signature (TS) described in doi: 10.1136/gutjnl-2014-308483 (ClinicalTrials.gov: NCT01657695).

Method: 309 (80% males) consecutive patients with HCC underwent US-guided liver biopsy for histological diagnosis, TS and miRNA evaluation (qRT/PCR), and P27 immunohistochemistry at HCC first diagnosis and at recurrence. Physicians performing treatment were blinded to TS status. Outcome was matched with TS presence only after end of follow-up.

Results: 83% of entire cohort underwent at least 1 treatment (14% systemic; 68% all others). Overall median survival was 21 months (M±SD: 28±23 months). TS**+** patients (27.7% of all) had significantly worst median survival (TS**+** vs. TS**–**: 12 vs. 41 months; p < 0.0001), independently from undergoing any treatment (TS**+** vs. TS**–** : median survival: 19 vs. 44 months; p < 0.0001) or undergoing supportive therapy only (median survival: 6 vs. 22 months; p < 0.0001). When evaluating outcome of cumulative therapies, a significantly different impact of the diverse therapies was present in TS**–** HCCs (survival for resection/LT significantly higher than that for RF and for TACE, the latter having the worst survival, p = 0.005) but not for TS**+** HCCs (various therapies: p = 0.641)(Fig. 1 a & b). For all types of treatment, survival was lower in TS**+** HCCs. Extremely rapid course of disease prevented LT in all but 4 TS**+** HCCs. As an unexpected side effect of the procedures, a shift from TS**–** to TS**+** HCC was demonstrated in sequential biopsies of recurrent HCCs undergoing TACE but not RF or resection (Figure 1c). Shift was associated with decreased survival and with consensual up-regulation of a set of MiRNA linked with angiogenesis, proliferation, cell cycle progression and with p27nuclear/cytoplasmic translocation.

Conclusion: HCCs bearing the transcriptomic signature have an extremely aggressive clinical course that ultimately impacts on survival despite the application of all the available treatments for HCC. TACE-associated shift from TS– to TS+ HCC can explain worse TACE outcome in HCC patients and demonstrates that transcriptomic signature is a dynamic feature, liable to be negatively influenced by therapeutic intervention.

Figure:



PB01-02 Quantitative magnetic resonance imaging predicts individual future liver performance after liver resection for cancer

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Background and aims: The future liver performance (FLP) of an individual undergoing surgical liver resection to remove cancer is critical for their survival and recovery. We report the development and clinical testing of a novel magnetic resonance image (MRI) post-processing tool that combines quantitative multiparametric MRI with anatomical liver segmentation to estimate FLP. This is intended to inform the assessment of individualised operative risk and augment patient and surgeon decision making prior to liver resection.

Method: This software combines iron-corrected T1 (cT1) mapping, previously demonstrated to correlate with fibroinflammation and predict clinical outcomes in chronic liver disease, with a 3D U-net pipeline to delineate the liver volume followed by semi-automatic delineation of Couinaud segments based on anatomical landmarks. Interactive removal of these segments, along with any interactively-defined virtual wedge resections, allows accurate estimation of the future liver remnant (FLR) volume, which when combined with quantitative cT1 mapping, provides a prediction of FLP, termed the “HepaT1ca score”. The ability of this score to predict post-operative morbidity, length of stay and regenerative capacity was evaluated in a prospective clinical trial (ClinicalTrials.gov NCT03213314).

Results: Of the 143 patients recruited, 135 underwent liver resection. 84% of participants had liver metastases from colorectal cancer, with the remaining having primary liver cancer or other secondary cancers. 21% of participants had cT1 values above the upper limit of normal (795ms) indicating increased risk of background liver disease. The HepaT1ca score showed a significant linear correlation with the modified Hyder-Pawlik score, an indicator of post-operative morbidity (adjusted $R^2 = 0.26$, $P < 0.001$), and liver regenerative performance (adjusted $R^2 = 0.46$, $P < 0.001$). Furthermore, in patients with an FLR below 90%, a high mean cT1 (> 795ms) was associated with a longer duration of hospital stay (median (IQR) of 6.5 (5.3-12) vs. 5 (4-7.1); $P = 0.0053$). cT1 also correlated with histological measures of inflammation and ballooning.

Conclusion: We demonstrate the utility of a non-invasive quantitative MRI approach for predicting post-operative liver performance. This has the potential to transform surgical decision-making and augment individualised risk assessment for patients undergoing liver resection for cancer.

Figure:

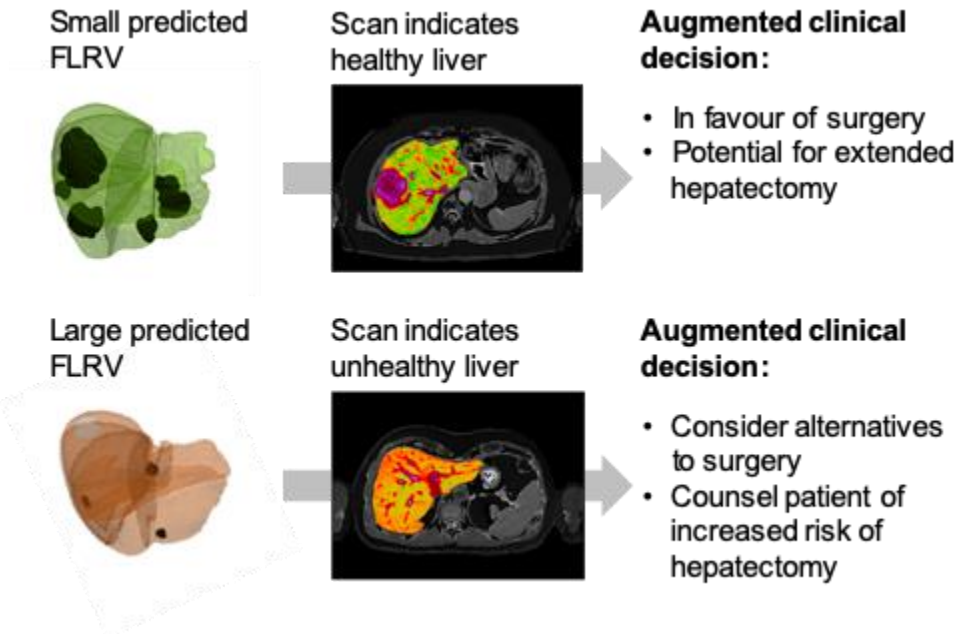

Figure: Concept diagram showing use of quantitative MRI in a clinical workflow highlighting exemplar cases from the HepaT1ca study.

PB01-03-YI Specific RNA profiles in serum and urine extracellular vesicles of patients with cholangiocarcinoma mimicking tumor tissue and cell expression: A novel liquid biopsy approach

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Background and aims: Cholangiocarcinoma (CCA) includes a heterogeneous group of biliary cancers with dismal prognosis. The etiology of the majority of CCAs is unknown, but some risk factors predispose its development, including the presence of primary sclerosing cholangitis (PSC). Simultaneously, 80% of patients with PSC have concomitant inflammatory bowel disease, mainly ulcerative colitis (UC). There are no accurate non-invasive biomarkers for the early diagnosis of CCA. In this sense, extracellular vesicles (EVs) have been postulated as a source of biomarkers and potential mediators of pathogenesis in several diseases. Therefore, we here investigated the potential value of serum and urine EVs as carriers of RNA biomarkers for the diagnosis of CCA and their potential involvement in disease pathogenesis.

Method: Serum and urine EVs were isolated from patients with UC (n=8 and 12), PSC (n=4 and 4) and CCA (n=10 and 17), as well as from healthy individuals (n=9 and 5) by differential ultracentrifugation. EV characterization was carried out by transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA) and immunoblot. The RNA profile of EVs was determined by microarray-based transcriptomics (Illumina). The diagnostic capacity of identified transcripts (AUC, sensitivity, specificity) was calculated using IBM SPSS statistics. Finally, the expression of the selected candidates was evaluated in human CCA tumor and surrounding healthy tissue from two independent cohorts of patients (TCGA and Copenhagen), as well as in cultures of normal human (NHC) and tumor (EGI-1 and TFK-1) cholangiocytes and in EVs released from those cell types.

Results: Isolated serum and urine EVs showed a rounded morphology (TEM), similar size (~180 nm in diameter, NTA) and typical EV markers (CD9, CD63 and CD81) by immunoblot. The transcriptomic analysis of these EVs presented differential RNA profiles in patients with CCA compared to healthy individuals or patients with other diseases (PSC and UC); some of them presenting the highest diagnostic capacity (AUC=1). In patients with CCA, a total of 268 transcripts in serum and 50 in urine EVs presented a differential abundance compared to healthy and PSC-UC. Importantly, 179 and 28 of those RNA transcripts showed the same differential expression in CCA tumor tissue (TCGA and Copenhagen) compared to normal surrounding liver tissue and normal human bile ducts. Moreover, 49 and 27 of those RNAs were also similarly deregulated in CCA cells and CCA cell-derived EVs, respectively, which showed high diagnostic capacity (AUC=0.92 CCA vs Ctrl) and may be involved in disease pathogenesis (cell growth, cell cycle, DNA repair, metabolism).

Conclusion: Serum and urine EVs of patients with CCA contain specific transcriptomic signatures with high diagnostic capacity. These RNAs may at least in part be released by CCA cells, functioning as a new liquid biopsy approach.

PB01-04 Predicting survival after hepatocellular carcinoma resection using deep-learning on histological slides

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Background and aims: The development of computational pathology and artificial intelligence promises to improve and standardize histological analyses, and may facilitate the extraction of “hidden” morphological features of potential clinical relevance. In this study, we used two deep-learning algorithms based on digitized histological slides, to build models for predicting the survival of patients with hepatocellular carcinoma (HCC) treated by surgical resection.
Method: Two independent series of patients were investigated: a discovery set (Henri Mondor Hospital, number of patients=194, total of 390 whole image digitized slides (WSI)) used to develop our algorithms with cross-validation and an independent validation set (TCGA, number of patients=328, total of 342 WSI).
The WSI were first divided into small squares, called "tiles", and features were extracted from these tiles with a pretrained convolutional neural network during the preprocessing step. During model development, the tiles were fed into the networks architectures. The first deep-learning based algorithm (designated as “SCHMOWDER ”) uses an attention mechanism on tumoral areas annotated by a pathologist while the second does not require human expertise.

Results: In the discovery set, c-indexes for survival prediction of SCHMOWDER and CHOWDER reached 0.78 and 0.75, respectively. Both models outperformed a composite score incorporating all baseline clinical, biological and pathological variables associated with survival. The prognostic value of the models were further validated in the TCGA dataset (c-indexes of 0.70 and 0.68 for SCHMOWDER and CHOWDER, respectively). As observed in the discovery series, both models had a higher discrimatory power than a score combining all relevant baseline variables associated with survival. Finally, pathological review showed that the tumoral areas most predictive of poor survival were characterized by vascular spaces, the macrotrabecular architectural pattern and a lack of immune infiltration.

Conclusion: Our study shows that artificial intelligence-based models using digitized histological slides predict overall survival after hepatocellular carcinoma resection more accurately than classical clinical, biological and pathological features. The analysis of areas classified as “low-risk” and “high-risk” by our model provides insight into the biological features that underly tumor aggressiveness.

PB01-05 miR-21 is increased in patients with NASH-associated HCC and contributes to hepatocarcinogenesis in mice with NAFLD

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Background and aims: Molecular mechanisms governing the progression of NASH towards HCC remain elusive. We have recently shown that concomitant miR-21 ablation and FXR activation prevent NASH development in mice. Here, we aimed to evaluate the role of miR-21-dependent signalling in NASH-associated carcinogenesis.

Method: miR-21 expression was evaluated in liver biopsies and surgically resected tumors from two independent patient cohorts, which included obese patients with NAFLD (n=200; SS cohort), HCC (n=362) and NASH-associated HCC (n=19) (TCGA cohort). WT and miR-21 KO mice were fed either a choline-sufficient, amino acid-defined control diet (CSAA; n=28) or a choline-deficient, amino acid-defined diet (CDAA; n=28) for 32 and 66 weeks. Liver samples were processed for histological analysis. miR-21 and dowstream targets, pro-inflammatory/pro-fibrogenic cytokines and metabolic relevant genes were also measured. A profiler PCR array was used to evaluate expression of liver cancer-related genes.

Results: In patients, miR-21 expression increased with NAFLD severity (steatosis, lobular inflammation, ballooning, fibrosis and the NAS score) in the SS cohort. Levels of miR-21 were markedly increased in the tumour tissue of both HCC and NASH-HCC patients compared with surrounding liver (TCGA cohort), and correlated with histological HCC markers (AFP, GPC3), disease stage and worse overall survival. WT mice fed the CDAA diet for 32 weeks developed NASH and fibrosis while CDAA-fed miR-21 KO mice exhibited increased activation of PPARα target genes and augmented mitochondrial activity. After 66 weeks, all WT mice on the CDAA diet had developed at least one preneoplastic nodule (~5.2 nodules/animal), with one animal developing trabecular HCC. miR-21 expression was significantly increased in CDAA-fed mice and further increased in HCC, concomitantly with decreased expression of PPARα. Livers presented hyperplastic foci, anisokaryosis as well as phenotypically altered and highly proliferative hepatocytes. Increased levels of pro-inflammatory/fibrogenic markers were particularly evident in pre-neoplastic liver tissues, alongside activation of oncogenic pathways. Strikingly, CDAA-fed miR-21 KO mice displayed serum ALT levels similar to control animals. The NAS score (<5), number of liver nodules (~2.3 nodules/animal), hepatocyte profliferation and expression of pro-inflammatory/fibrogenic markers and oncogenes were all significantly reduced, comparing with CDAA WT-fed mice.

Conclusion: Activation of miR-21-dependent pathways appears to contribute to NAFLD progression up to NASH-associated carcinogenesis, with its inhibiton halting HCC development. Targeting miR-21 may constitute an appealing therapeutic approach to ameilorate NASH and its progression towards HCC. (PTDC/MED-PAT/31882/2017, FCT, PT and EU H2020 Marie Sklodowska-Curie 722619 grant).

PB02-01-YI Hepatic RIPK3 signalling differentially modulates lipid metabolism and inflammation/carcinogenesis in non-alcoholic steatohepatitis

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Background and aims: Necroptosis embodies an inflammatory cell death pathway dependent on receptor-interacting-protein kinase 3 (RIPK3) activity that may influence hepatocellular damage in non-alcoholic fatty liver disease (NAFLD). A signalling interplay between necroptosis and lipid metabolism is emerging. We aimed to evaluate the impact of RIPK3 signalling in the pathogenesis of NAFLD and progression towards cancer.

Method: RIPK3 levels were evaluated in two independent cohorts of morbidly obese patients with biopsy-proven diagnosis of NAFLD (cohort A: n = 146; cohort B: n = 71), and correlated with clinical and biochemical parameters. C57BL/6 wild-type (WT) or RIPK3-deficient (*Rip3k*$^{-/-}$) mice were fed a choline-deficient L-amino acid-defined diet (CDAA; n=14) or a control choline-sufficient L-amino acid-defined diet (CSAA; n=14) for 32 and 66 weeks. Liver samples were processed for histological and biochemical analysis of damage, inflammation and fibrosis. Lipidomic analysis was performed and a profiler PCR array was used to evaluate expression of liver cancer-related genes.

Results: In both NAFLD cohorts, RIPK3 levels were increased in patients with more advanced disease, correlating with hepatic collagen-1α1, inflammatory and necroptosis-related proteins, and circulating transaminases. In mice, RIPK3 deficiency ameliorated CDAA-induced inflammation and fibrosis. Intriguingly, *Rip3k*$^{-/-}$ mice displayed increased liver fat accumulation, body weight gain and circulating insulin levels, irrespective of the diet, when compared with WT mice. Lipidomics showed that deletion of RIPK3 shifted hepatic lipid species profiles, impacting on acyl chain length and saturation. In line with these metabolic changes, peroxisome proliferator-activated receptor-γ (PPARγ) was increased in the liver of *Rip3k*$^{-/-}$ mice. Finally, *Rip3k*$^{-/-}$mice on the CDAA diet for 66 weeks displayed reduced incidence of macroscopic preneoplasic nodules, accompanied by significantly reduced Ki67 positive hepatocytes. Indeed, microarray profiling and subsequent gene and protein expression analyses showed that the absence of RIPK3 hampered tumorigenic signalling pathways, such as cell cycle progression, resistance to cell death and epithelial-mesenchymal transition process.

Conclusion: Hepatic RIPK3 correlates with NAFLD severity in humans and mice. RIPK3 plays an opposing role in controlling steatosis versus inflammation/carcinogenesis in CDAA-fed mice, suggesting that these two phenomena are dissociated events in NAFLD.

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PB02-02 SBRT vs TAE/TACE in Hepatocellular carcinoma: results from a Phase III trial (NTC02323360)

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Background and aims: In unresectable HCC patients with intermediate-stage disease, transcatheter arterial embolization (TAE) +/- co-administration of arterial chemotherapy (TACE) has shown partial responses in 15–55% of cases, and significantly delays tumor progression and vascular invasion. However while repeated courses of TAE/TACE can be administered in case of incomplete response, alternative treatment options may be considered to maximize local control. Stereotactic body radiotherapy (SBRT), delivering very high doses in a limited number of fractions in a highly conformal manner, is an emerging treatment option for radical treatment of inoperable HCC and may be proposed in case of local relapse following one or more TAE/TACE courses.
Method: This is a multicentre, prospective, randomised controlled, unblinded, parallel-group superiority trial of SBRT versus standard TAE/TACE for the curative treatment of intermediate stage of HCC after incomplete reponse following one TAE/TACE cycle (NCT02323360). Primary endpoint was Local Control (LC). Secondary endpoints were Progression Free-Survival (PFS), Overall Survival (OS) and incidence of acute and late complications. In order to detect an HR=0.18 (which translates in 45% difference at the analysis time) with a power of 80% at 5% 2 sided of the log-rank test , 18 events (approximately 50 patients, 25 per arm) are needed. A preliminary analysis was performed when the preplanned number of events (n=20) was reached.
Results: At the time of our analysis 40 patients were enrolled, 19 (49%) in the TAE/TACE and 21 (51%) in the SBRT arm respectively. Median age was 75 (range 52-86) years. All patients received at least ≥ 1 TAE/TACE course prior to enrolment; no significant differences were found between the 2 arms with regard to prior resection (n=8, 20%), Radiofrequency Ablation (RFA, n=14, 35%) or Percutaneous Ethanol Injection (PEI, n=4, 10%). Median follow-up was 18 months (range, 2-56 months). Patients were classed stage A and B according Barcelona Clinic Liver Cancer (BCLC) staging system in 7 (18%) and 33 (82%) respectively. One and 2-year LC rates were 57% and 36%, with a median LC of 12 months (CI95% 16-33 months). Use of SBRT resulted in superior LC as compared to TAE/TACE (median not reached versus 8 months, p=0.0002). PFS was 29% and 16% at 1 and at 2 years, respectively. At the event of progression, 8 patients in the SBRT arm received a further TAE/TACE administration while 10 patients in the TAE/TACE arm received SBRT. OS was 96% and 90% at 1 year and at 2 years, respectively. No grade ≥3 toxicity was recorded, and no differences in overall toxicities were found between the 2 arms.
Conclusion: In patients affected by unoperable HCC experiencing incomplete response following ≥1 cycle of TAE/TACE, SBRT was correlated to significantly higher LC rates as compared to rechallenge with TAE/TACE

PB02-03-YI Inhibiting endoplasmic reticulum stress in hepatic stellate cells decreases the progression of hepatocellular carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) is a primary liver tumor that usually occurs in the background of liver cirrhosis. Activated stellate cells play a key role in the pathogenesis of HCC. They are the main source of pro-fibrotic signaling and produce growth factors that actively fuel tumor cell proliferation and migration. In this study, we aimed to identify the role of ER-stress in the cross-talk between stellate cells and cancer cells in HCC.
Method: Mice were injected once per week with diethylnitrosamine to induce HCC with underlying liver cirrhosis. Mice were treated with an IRE1a inhibitor 4u8C or control, samples were taken after 25 weeks. Tumor burden and collagen deposition was quantified on H&E and Sirius red staining, respectively. To study the interactions between stellate cells and tumor cells in vitro, HCC-cell lines (HepG2 and Huh7) and stellate cell line (LX2) were co-cultured using transwell assays, 2D co-cultures, 3D spheroids and cell-engrafted human liver 3D-scaffolds. Chemotaxis was assessed on fluorescently labelled cells using scratch wound assays and 2D CellDirectors, respectively. Images from biopsies from patients with HCC stained with antibodies against WIPI1, SHC1, PPP2R5B and BiP were obtained through the Human Protein Atlas. Gene expression profiles of HCC with and without a fibrous stroma was accessed through PubMed's Gene Expression Omnibus, and a gene-set enrichment assay was performed.
Results: Treatment with 4u8C lead to a significant decrease in size and number of HCC in vivo. Sirius red staining showed a significant decrease in collagen deposition after treatment with 4u8C, compared to controls. In vitro experiments showed that tumor cells secrete factors that cause hepatic stellate cells to undergo endoplasmic reticulum (ER)-stress, which contributes to their activation. These activated stellate cells induce the progression of HCC by promoting tumor cell proliferation and migration. Inhibiting IRE1a-ribonuclease activity with 4u8C or siRNA-transfection decreased stellate cell activation, which inhibited tumor cell proliferation and migration in vitro. A gene-set enrichment assay revealed an increase of genes involved in ER stress in fibrotic samples of HCC patients. Immunohistochemistry on HCC patient-liver biopsies confirmed the presence of ER stress markers in the fibrotic scar tissue and near hepatic blood vessels.

Conclusion: These results suggest that IRE1a could play an important role in mediating the cross-talk between stellate cells and cancer cells. Components of the ER-stress pathway may be therapeutically relevant for treating patients with HCC.

PB02-04 Clinical activity of cabozantinib in patients with advanced hepatocellular carcinoma previously treated with anti-VEGF and immuno-oncology therapy: Subgroup analysis from the phase 3 CELESTIAL trial

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Background and aims: The treatment landscape for advanced hepatocellular carcinoma (aHCC) is anticipated to evolve rapidly with immuno-oncology (IO)-based regimens likely to become a standard of care option for treatment-naïve patients; however, little data are available to guide sequencing following these therapies. Cabozantinib (C), a multikinase inhibitor, has shown promising activity following IO agents in advanced renal cell carcinoma. For aHCC, C improved overall survival (OS) and progression-free survival (PFS) vs placebo (P) in patients (pts) previously treated with sorafenib in the phase 3 CELESTIAL trial. Unlike other pivotal phase 3 trials in this setting, CELESTIAL allowed up to 2 prior regimens for aHCC. In this exploratory analysis, we assess the clinical activity of C in pts in CELESTIAL who received prior sorafenib and IO therapy.

Method: As of June 2017, 707 pts were randomized 2:1 to C (60 mg QD) or P. Eligible pts were Child-Pugh A, ECOG PS 0-1, must have received prior sorafenib, and could have received up to 2 prior regimens for aHCC. Outcomes were evaluated for pts who received prior IO therapy, using a data cutoff of December 2017 to allow for extended follow-up.

Results: 130 pts in the C arm and 62 pts in the P arm received 2 prior regimens for aHCC; among these pts, 14 in the C arm and 3 in the P arm received prior IO therapy as well as the required sorafenib. In the C arm, 9 pts had received nivolumab (2 in combination with ipilimumab), 4 had received pembrolizumab, and 1 had received durvalumab. In the prior IO subgroup, median OS with C was 7.9 months (mo) (95% CI, 5.1-not estimable), and median PFS was 3.7 mo (95% CI, 1.9-5.6), while in the overall 2 prior regimen subgroup, median OS with C was 8.5 mo (95% CI, 7.4-9.7), and median PFS was 3.7 mo (95% CI, 3.3-4.1). In the prior IO subgroup, the median duration of exposure to C was 3.7 mo (range 1.9-18.7), 9 pts (64%) experienced a grade 3/4 adverse event (AE), and 1 pt (7%) discontinued due to a treatment-related AE. In the overall 2 prior regimen subgroup, the median duration of exposure to C was 3.7 mo (range 0.5-23.9), 85 pts (66%) experienced a grade 3/4 AE, and 19 pts (15%) discontinued due to a treatment-related AE.

Conclusion: Pts with aHCC previously treated with anti-VEGF and IO therapy had similar outcomes on C to other pts who received 2 prior regimens, suggesting clinical activity and tolerability of C in pts previously treated with IO.

PB02-05 Pattern of progression in advanced hepatocellular carcinoma treated with ramucirumab/placebo: Results from two randomised phase 3 trials (REACH/REACH-2)

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Background and aims: REACH (NCT01140347) and REACH-2 (NCT02435433) studied ramucirumab (RAM) in patients (pts) with advanced hepatocellular carcinoma (HCC) following sorafenib; REACH-2 enrolled pts with baseline alpha-fetoprotein (AFP) ≥ 400 ng/mL, and met its primary endpoint of overall survival (OS) for RAM versus (vs) placebo. This post-hoc analysis examined radiological progression patterns (RPP) incidence every 6 weeks per RECIST v1.1, and if RPP were related to overall survival (OS) and post-progression survival (PPS).

Method: Pts with advanced HCC, Child-Pugh A and ECOG PS 0-1 with prior sorafenib were randomized (REACH 1:1; REACH-2 2:1) to receive RAM 8 mg/kg or placebo Q2W. Among pts with ≥1 RPP (new extrahepatic lesion [NEH], new intrahepatic lesion [NIH], extrahepatic growth [EHG] or intrahepatic growth [IHG]), results were analysed by trial and for pooled individual patient data of REACH-2 and REACH (AFP ≥ 400 ng/mL). Cox models evaluated treatment effect of RPP on OS, and prognostic implications of RPP on OS (adjusting baseline ECOG PS, AFP, macrovascular invasion, arm) and on PPS (adjusting ECOG PS, AFP at progression).

Results: RPP incidence in the pooled population was NEH 39 %; NIH 24 %; EHG 39 %; and IHG 37 %. When examining NEH vs other RPP, PPS was worse among those with NEH in REACH (hazard ratio [HR] 2.33, 95 % confidence interval [CI] 1.51, 3.60), REACH-2 (HR 1.49, 95 % CI 0.72, 3.08), and the pooled population (HR 1.75, 95 % CI 1.12, 2.74). Use of post-discontinuation therapy may have influenced results. OS was also significantly reduced in those with NEH across studies (Table). RAM provided OS benefit in the pooled population, including pts with NEH (HR 0.56, 95 % CI 0.39, 0.80).

Conclusion: Acknowledging limitations of post-randomization RPP analysis, the emergence of NEH on RAM or placebo may be an independent poor prognostic factor for PPS. The impact of RAM on OS was consistent across all RPP subgroups.

Figure: Multivariate Cox Models (HR [95 % CI]) of OS by RPP

	REACH	REACH-2	Pooled (AFP ≥ 400 ng/mL)
RPP Pattern vs All Others	**N=414**	**N=211**	**N=398**
NEH	**1.84 (1.24, 2.73)**	**1.94 (1.05, 3.60)**	**1.89 (1.27, 2.83)**
NIH	1.10 (0.73, 1.66)	1.55 (0.67, 3.58)	1.24 (0.76, 2.02)
EHG	1.08 (0.75, 1.55)	1.31 (0.71, 2.43)	1.12 (0.75, 1.67)
IHG	1.08 (0.75, 1.57)	1.68 (0.95, 2.97)	1.48 (1.01, 2.16)

INDUSTRY

Industry satellite symposia

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Friday 7 February 2020, 13:00-14:00 – Room: Zenit

The combination revolution in hepatocellular carcinoma
Chair: Andrew Zhu, *United States*

13:00 - 13:05	Welcome and introductions Andrew Zhu, *United States*
13:05 - 13:15	Exploring the rationale for combination therapy in HCC Josep Llovet, *United States*
13:15 - 13:30	Extending survival: latest phase III data for advanced HCC Andrew Zhu, *United States*
13:30 - 13:50	Navigating a multi-disciplinary approach to HCC treatment decisions Q&A Riccardo Lencioni, *Italy* Panelists: Peter Galle, Riccardo Lencioni, Josep Llovet Moderator: Andrew Zhu, *United States*
13:50 - 14:00	Evolving treatment guidelines and clinical practice for HCC Peter Galle, *Germany*
14:00	Meeting close Andrew Zhu, *United States*

ELI LILLY

Friday 7 February 2020, 19:00-20:00 – Room: Zenit

Current perspectives on the management of advanced HCC
Chair: Bruno Sangro, *Spain*

19:00 - 19:05	Welcome and Introduction (housekeeping) Bruno Sangro, *Spain*
19:05 - 19:45	Current perspectives on the practical management of advanced HCC – patient case presentation and discussion Ian Chau, *United Kingdom* Panelist: Bruno Sangro
19:45 - 19:55	Sequencing treatments in advanced HCC: consensus and controversy What's in it for my patient? Bruno Sangro, *Spain*
19:55 - 20:00	Closing notes Bruno Sangro, *Spain*

Company profiles

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