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ABSTRACT BOOK
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INDUSTRY

LATE-BREAKER ABSTRACT PRESENTATIONS

LBO-01 Exploiting senescence for the treatment of liver cancer

Cun Wang[1 2], Serena Vegna[3], Haojie Jin[1 2], Wenxin Qin[1], Leila Akkari[3], Rene Bernards[2]
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Background and aims: Liver cancer is the fourth leading cause of cancer death worldwide and remains difficult to treat due to a paucity of drugs that target critical dependencies. Broad spectrum kinase inhibitors like sorafenib, regorafenib, and lenvatinib provide only modest survival benefit to hepatocellular carcinoma (HCC) patients and biomarkers of response are still lacking. We and others have recently hypothesized that induction of senescence represents a promising strategy for the treatment of cancer, especially when such pro-senescence therapy is combined with a second drug that selectively kills senescent cancer cells (senolysis).
Method: Through a kinome-centered genetic screen for genes required for proliferation in liver cancer cells, followed by a second compound screen based on senescence detection, potential pro-senescence effectors in liver cancer cells were identified. We further sought to identify novel compounds that selectively kill senescent liver cancer cells. We screened a library consisting of 260 compounds targeting GPCRs in both proliferating and XL413-treated senescent Huh7 cells. Finally, we investigated the potential of this therapy in both xenografts and immune-competent liver cancer mouse models.
Results: We report here that pharmacological inhibition of the DNA replication kinase CDC7 induces senescence selectively in *TP53* mutant liver cancer cells. We find that HCC cells rendered senescent through treatment with CDC7 inhibitor are selectively killed by the anti-depressant sertraline. Transcriptome sequencing of senescent HCC cells shows that sertraline suppresses mTOR signaling and inhibition of this pathway with more selective drugs is highly effective in causing apoptotic cell death of senescent HCC cells. Mechanistically, we find that in CDC7-inhibitor induced senescence, feedback re-activation of mTOR through receptor tyrosine kinase signaling is blocked, leading to sustained inhibition of signaling following treatment with mTOR inhibitor. We show in multiple *in vivo* liver cancer models that a combination of CDC7 and mTOR inhibitors results in dramatic tumor growth inhibition, superior to either single agent effects.
Conclusion: Our data suggest that a pro-senescence therapy combined with a senolytic drug could be an effective treatment for liver cancer.

LBO-02 A computational model for optimizing immunotherapy strategies in HCC based on early treatment response dynamics

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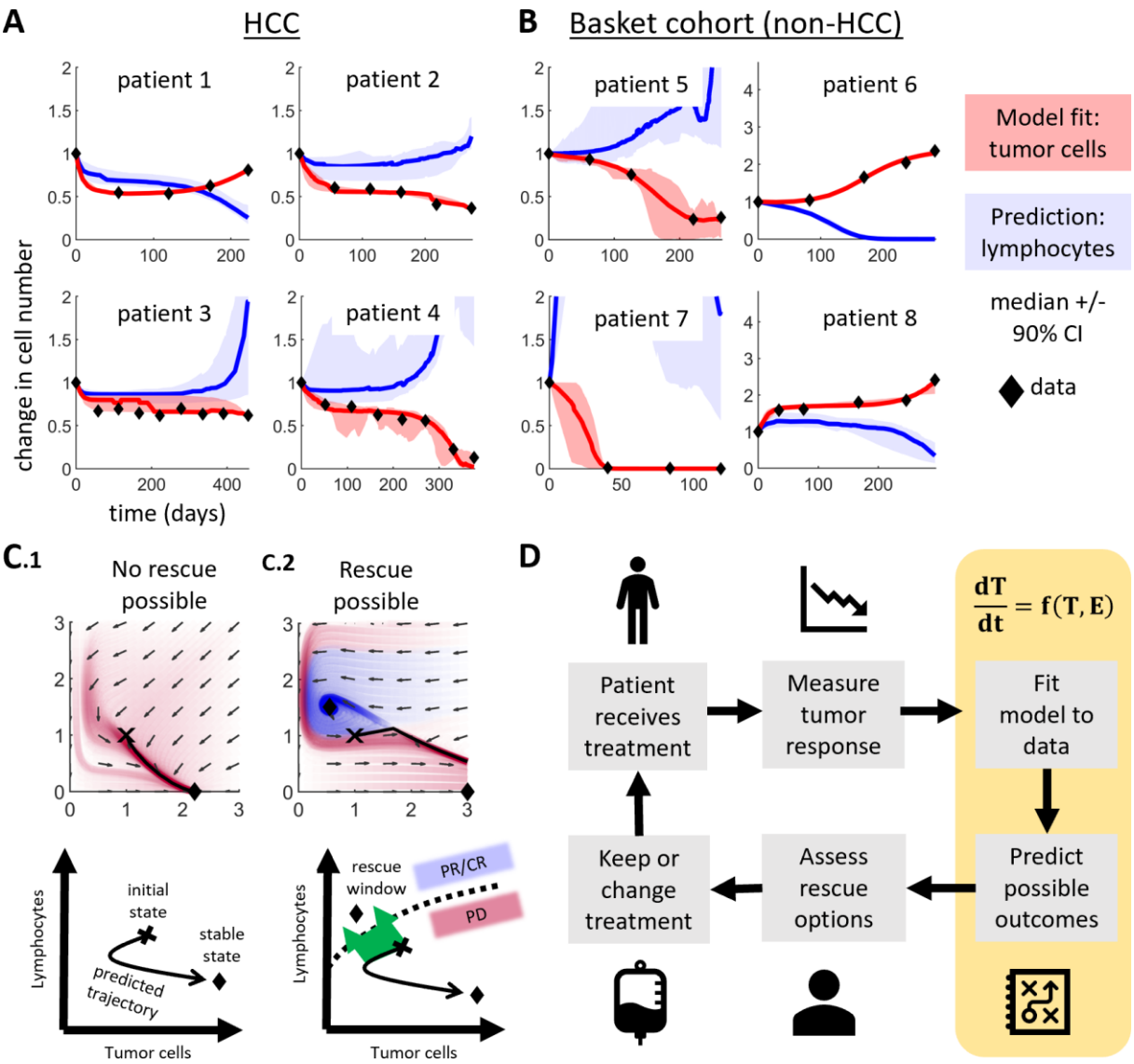
Background and aims: As immunotherapy and new targeted treatment options have become available, the systemic treatment of hepatocellular carcinoma (HCC) is undergoing fundamental changes. In this context, identifying non-responders early during treatment and finding optimal second-line or additional rescue strategies for these patients is a key challenge. Because of the vast number of possible drug combinations and treatment sequences, clinical testing of all options is not feasible and alternative approaches to optimize treatment strategies are needed.

Method: We used a patient-specific model of cellular interactions in human solid tumors that can answer these questions by computational simulation applied to early treatment response data from human cancer patients undergoing immunotherapy. The model is expressed as a system of two ordinary differential equations (ODEs) with four global and three patient-specific parameters. It explicitly tracks tumor cells and lymphocytes in the tumor microenvironment (TME). The model was fitted to clinical data of tumor volume changes over time in a patient-specific way. Via a phase analysis, this model could predict stable states (attractors) and identify patients with a rescue window.

Results: We applied this model to tumor volume timelines of patients in a basket cohort of non-HCC solid tumors (N = 101) and a cohort of HCC patients (N = 37) during treatment with immune checkpoint inhibitors. These data were publicly available in previously published clinical trials. We show that this model can (a) recapitulate the response kinetics to immunotherapy (median R^2 >0.90), (b) predict progression free time at 1.3 years (Cox hazard ratio 0.195, p <0.005 for the pooled cohort) based on three initial measurements of tumor volume change and (c) identify non-responding patients with a “rescue window” who could benefit from combinatorial treatment strategies.

Conclusion: Computational modeling can provide a tool to predict treatment response and optimize treatment strategies in HCC and other solid tumors. Our next aim is to implement this approach in adaptive clinical trials in which treatment strategies are optimized according to early treatment response dynamics.

Figure: (A) Median model fit with 90% confidence interval (CI) for HCC data, (B) Model fit for non-HCC data (representative patients), (C) Phase space analysis with stable states (attractors) for each patient, shown for two patients. (C.1) This patient only had one “progressive disease” attractor and converged to this point over time. (C.2) Another patient had two attractors and thus, two basins of attraction. One of these basins converged to a “response” attractor, suggesting that these patients could be rescued by shifting their TME to a more favorable state. (D) Envisioned clinical workflow enabled by the model.


A
HCC
B
Basket cohort (non-HCC)
patient 1
patient 2
patient 3
patient 4
patient 5
patient 6
patient 7
patient 8
change in cell number
time (days)
Model fit: tumor cells
Prediction: lymphocytes
median +/- 90% CI
data
C.1
No rescue possible
C.2
Rescue possible
Lymphocytes
Tumor cells
initial state
stable state
predicted trajectory
rescue window
PR/CR
PD
D
Patient receives treatment
Measure tumor response
Fit model to data
Predict possible outcomes
Assess rescue options
Keep or change treatment
dT/dt = f(T, E)

LBO-03 Dual targeting of G9a and DNM-methyltransferase-1 for the treatment of experimental cholangiocarcinoma

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Email:

Background and aims: Cholangiocarcinoma (CCA) is a deadly disease usually diagnosed at advances stages when treatment options are limited. CCAs are histologically and molecularly heterogeneous tumors highly resistant to systemic therapies, and targeted drugs are yet to prove their efficacy. Identification of novel targets for CCA treatment is therefore necessary. Epigenetic alterations are increasingly recognized in CCAs which may constitute druggable targets. DNA and histone methylation reactions functionally cooperate in fostering tumor growth. We evaluated the therapeutic efficacy of a first-in-class substrate-competitive dual G9a H3K9-methyltransferase and DNA-methyltransferase 1 (DNMT1) inhibitor in different in vitro and in vivo CCA models
Method: G9a and DNMT1 mRNA and protein levels were examined in human CCA tissues by qPCR and immunohistochemistry. Dual targeting of G9a and DNMT1 was examined in CCA cells by combination treatment with the G9a and DNMT inhibitors BIX-01294 and decitabine. Anti-CCA efficacy of our G9a/DNMT1 inhibitor lead compound, CM-272, was tested in human CCA cells, alone and in combination with cisplatin, Mcl-1 or ErbB inhibitors. Microarray transcriptomic analyses were performed in two CCA cell lines treated with CM-272. CM-272 was tested in subcutaneous and orthotopic mouse xenografts of human CCA cells, and in a new model of cholangiocarcinogenesis (mice with JNK1/2 deletion in hepatocytes, JNKΔhepa)
Results: G9a and DNMT1 expression was increased in human CCA samples and cell lines compared to non-transformed tissues and cells. Combined treatment of CCA cells with BIX-01294 and decitabine resulted in synergistic growth inhibition. CM-272 showed GI50 values in the nanomolar range in six human CCA cell lines and markedly inhibited their colony formation capacity. CM-272 synergized with cisplatin, an Mcl-1 inhibitor, or the ErbB pathway inhibitors afatinib or lapatinib in the inhibition of CCA proliferation. CM-272 inhibited the growth of subcutaneous and orthotopic CCA xenografts, and the development of preneoplastic CCA lesions in JNK‑‑‑‑ mice. No systemic or hepatic toxicity were observed in CM-272 treated mice. Mechanistically, microarray analyses showed that CM-272 induced a strong metabolic reprogramming and interfered with growth factor signaling pathways in CCA cells
Conclusion: Pharmacologic interference with G9a and DNMT1 might be a promising strategy for the development of effective therapies against CCA

LBP-01 CDK12 inhibition mediates DNA damage and suppresses adaptive responses to sorafenib treatment in hepatocellular carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) is one of the most frequent malignancies and a major leading cause of cancer-related deaths worldwide. First-line therapeutic options like sorafenib and lenvatinib provide only modest survival benefit to HCC patients. This study aims to identify novel druggable candidates for HCC patients.
Method: A non-biased approach used to identify vulnerabilities of HCC cells was performed by screening a custom CRISPR library targeting all known human kinases. Whole-transcriptome sequencing (RNA-Seq) and bioinformatics analysis were performed to explore the mechanisms of the action of CDK12 inhibitor in HCC cells. Multiple *in vitro* and *in vivo* assays were conducted to determine the synergistic effects and the involved mechanism between the combination of CDK12 inhibition and sorafenib.
Results: We identify CDK12 as critically required for HCC cell lines. Suppression of CDK12 using shRNAs or its inhibition by the covalent inhibitor THZ531 leads to robust proliferation inhibition. THZ531 preferentially suppresses the expression of DNA repair-related genes and induces strong DNA damage response in HCC cell lines. The combination of THZ531 and sorafenib showed striking synergy in cell viability by inducing apoptosis or senescence. The synergy between THZ531 and sorafenib may derive from the mechanism that THZ531 impairs the adaptive responses induced by sorafenib treatment.
Conclusion: Our data highlight the potential of CDK12 as a drug target for HCC patients. The striking synergy of THZ531 and sorafenib may promote innovative combination therapies for this challenging cancer.

LBP-02 Attenuated XPO5-mediated export of pre-miRNA confers sorafenib resistance in HCC

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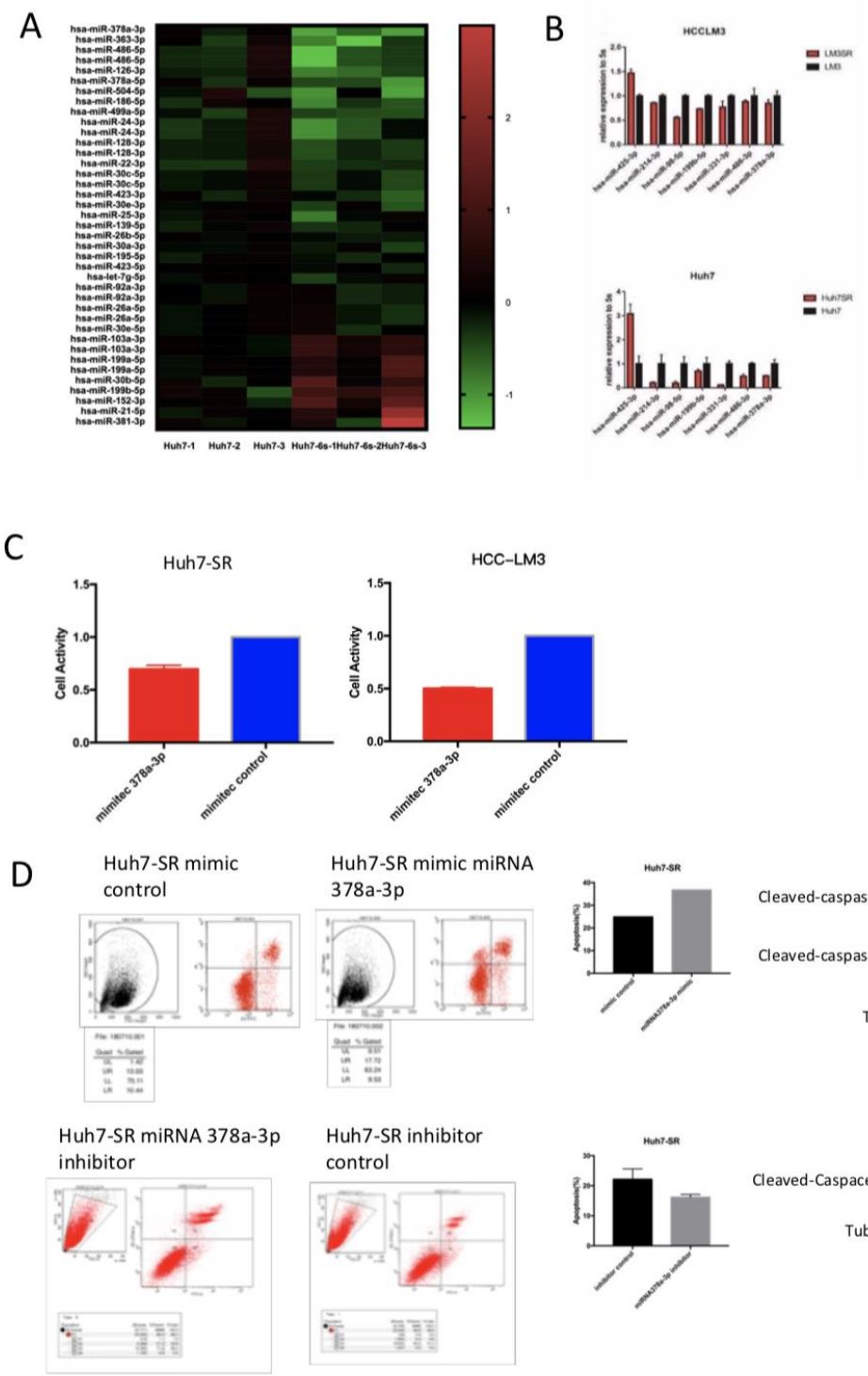
Background and aims: Sorafenib is the first-line treatment for advanced hepatocellular carcinoma (HCC), but the clinical response to sorafenib is seriously limited by drug resistance. Dysregulation of microRNA play a critical roles in sorafenib resistance. Our studies have a better understanding of the dysfunction of microRNA in the sorafenib resistance in HCC cells.
Method: We used FISH，separation of nuclear and cytoplasmic fractions, quantitative reverse transcription PCR to study the locality of the precursor microRNA-378a and immunohistochemistry, luciferase assays and immunoblotting to study the role of miR-378a in sorafenib resistance cell. Patient-derived xenografts and cell-derived xenografts were used to study the functions of microRNA-378a.
Results: Our miRNA microarray data indicate that MicroRNAs (miRNA) were mostly downregulated in sorafenib resistance cell, such as microRNA-378a. There was a significant uncoupling of primer microRNA and pre-miRNAs levels in sorafenib-resistant cells. Down-regulation of xpo5 caused the precursor-microRNA-378 exporting nucleus limitation and restrained the maturation of microRNA, which reduced microRNA-378a expression conferring sorafenib resistance to hepatocellular carcinoma cells by activating IGF-1R to regulate RAS/RAF/ERK signaling pathways. LXRα (Liver X receptor alpha) functioned as a transcription activator of microRNA-378a and made sorafenib-resistance cells and model sensitive to sorafenib.
Conclusion: Attenuated XPO5-mediated export of precusor-miRNA limitation confered sorafenib resistance in HCC. GW3965 treatment (LXRα agonist) in combination with sorafenib represents a novel therapeutic strategy for HCC treatment.

Figure:


A
hsa-miR-378a-3p
hsa-miR-363-3p
hsa-miR-486-5p
hsa-miR-486-5p
hsa-miR-126-3p
hsa-miR-378a-5p
hsa-miR-504-5p
hsa-miR-186-5p
hsa-miR-499a-5p
hsa-miR-24-3p
hsa-miR-24-3p
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hsa-miR-128-3p
hsa-miR-22-3p
hsa-miR-30c-5p
hsa-miR-30c-5p
hsa-miR-423-3p
hsa-miR-30e-3p
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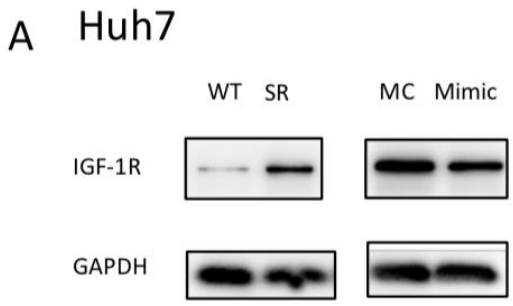
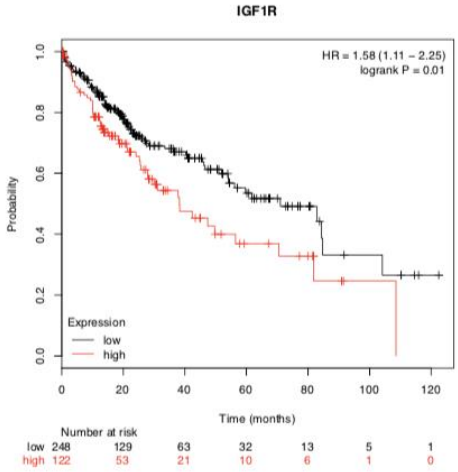
hsa-miR-30b-5p
hsa-miR-199b-5p
hsa-miR-152-3p
hsa-miR-21-5p
hsa-miR-381-3p
Huh7-1 Huh7-2 Huh7-3 Huh7-6s-1Huh7-6s-2Huh7-6s-3
2
1
0
-1
B
HCCLM3
LM3SR
LM3
Huh7
Huh7SR
Huh7
C
Huh7-SR
HCC-LM3
Cell Activity
1.5
1.0
0.5
0.0
mimitec 378a-3p
mimitec control
D
Huh7-SR mimic control
Huh7-SR mimic miRNA 378a-3p
Huh7-SR miRNA 378a-3p inhibitor
Huh7-SR inhibitor control
Huh7-SR
Cleaved-caspase-7
Cleaved-caspase-3
Tublin
Hu7 IC
Hu7 miR-378a-3p inhibitor
Cleaved-Caspace-3
Tublin
H7SR MC
H7SR miR-378a-3p mimic

A





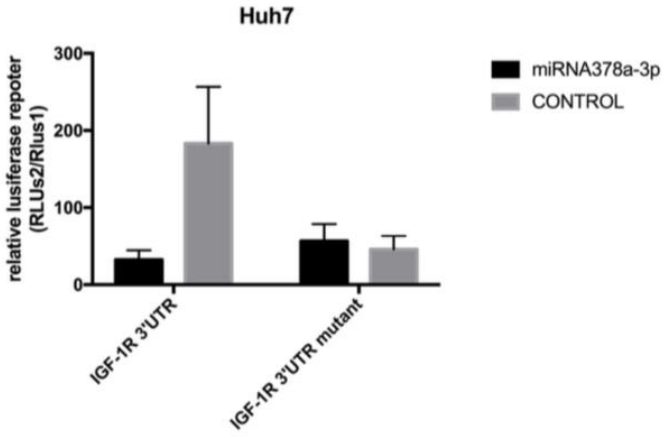
B

Position 5590-5596 of IGF1R 3' UTR	5' ...UCAAUAGGGCUCUUAAGUCCAGU...
hsa-miR-378a-3p	3' CGGAAGACUGAGGUUCAGGUCA

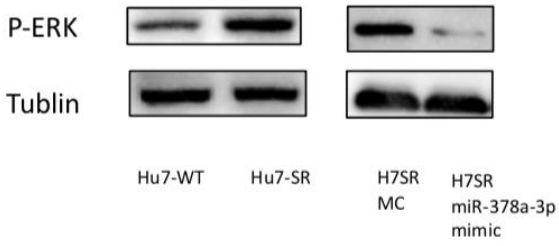
Wild type 3'UTR 5'...UCAAUAGGGCUCUUAAGUCCAGU...

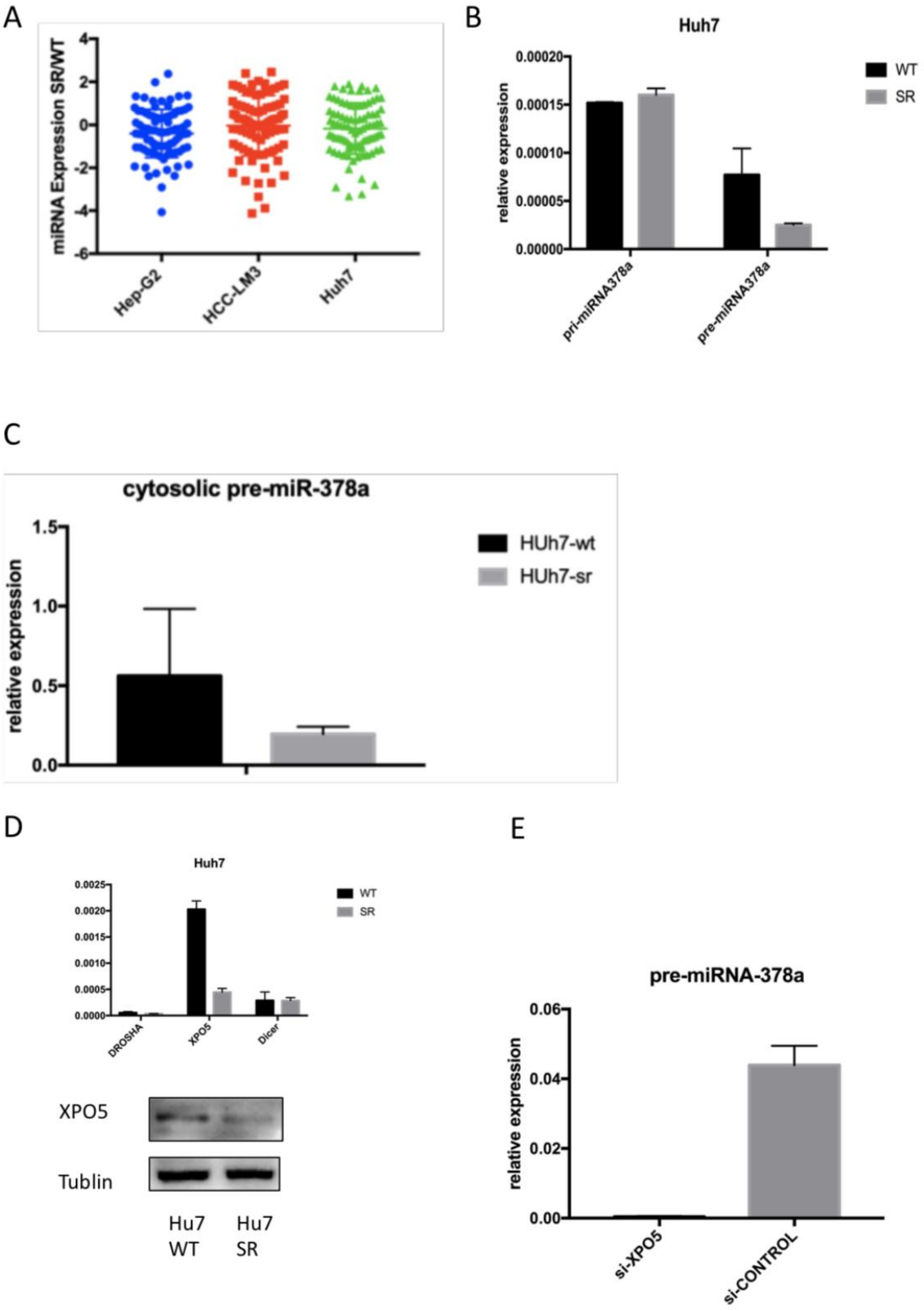
Has-miR-378a-3p 3'... CGGAAGACUGAGGUUCAGGUCA...

Mutant 3'UTR 5'...UCAAUAGGGCUCUUAAGUCGUCU...



C




A
miRNA Expression SR/WT
4
2
0
-2
-4
-6
Hep-G2
HCC-LM3
Huh7
B
Huh7
relative expression
0.00020
0.00015
0.00010
0.00005
0.00000
WT
SR
pri-miRNA378a
pre-miRNA378a
C
cytosolic pre-miR-378a
relative expression
1.5
1.0
0.5
0.0
HUh7-wt
HUh7-sr
D
Huh7
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
WT
SR
DROSHA
XPO5
Dicer
XPO5
Tublin
Hu7 WT
Hu7 SR
E
pre-miRNA-378a
relative expression
0.06
0.04
0.02
0.00
si-XPO5
si-CONTROL

LBP-03 The long non-coding RNA SNHG16 affects prognosis in hepatocellular carcinoma by regulating p62 expression

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Background and aims: Long non-coding RNAs (lncRNAs) regulate tumor development and progression by promoting proliferation, invasion and metastasis. The oncogenic role of lncRNA SNHG16 in hepatocellular carcinoma (HCC) has not been revealed.
Method: SNHG16 was identified by microarray analysis. Expression levels and prognostic information for SNHG16 were determined based on clinical samples. Colony formation assay, wound-healing assay, transwell assay, and flow cytometry assay were performed to determine the cell proliferation, migration, invasion and apoptosis. Then, we examined the possible roles of SNHG16 in xenograft mouse model. Dual luciferase reporter assay was conducted to evaluate the interactions among SNHG16, miR-17-5p and p62. Protein levels of key targets from the mTOR and NF-κB signaling pathways were estimated using western blotting.
Results: LncRNA SNHG16 is upregulated in HCC and correlates with poorer prognosis. Patients with high SNHG16 expression showed poorer overall and disease-free survival than patients with low SNHG16 expression. Multivariate Cox regression analysis revealed that SNHG16 expression is an independent predictor for poor overall and disease-free survival. SNHG16 was found to promote HCC cell proliferation, migration, and invasion; it also inhibited apoptosis *in vitro* and accelerated tumor development *in vivo*. We found SNHG16 to alter levels of miR-17-5p, which regulated expression of p62, which has been shown to regulate the mTOR and NF-κB pathways. Increasing or decreasing SNHG16 expression in HCC cells was shown to activate mTOR and NF-κB signaling.
Conclusion: These results reveal a potential mechanism for the oncogenic role of SNHG16 in HCC. SNHG16 may therefore be a promising novel marker for diagnosis and treatment of HCC.

LBP-04 Influence of sustained virological response in diagnostic performance of alpha-fetoprotein for hepatocellular carcinoma in patients with HCV-cirrhosis

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Background and aims: The usefulness of alpha-fetoprotein (AFP) for hepatocellular carcinoma (HCC) surveillance has been limited by its low sensitivity (sen.) and specificity (spe.), mainly in patients with viral cirrhosis. The aim of this study was to know AFP behavior in patients with HCV-cirrhosis included in an HCC surveillance program, comparing patients with sustained virological response (SVR) and viremic patients (VIR).
Method: 349 patients with HCV-cirrhosis, without HCC, follow-up ≥18 months and >3 AFP determinations
(det.) (223 SVR/126 VIR), enrolled in a surveillance program based in biannual US/AFP were analyzed. 105 additional patients with HCV-cirrhosis who developed HCC within the program were also included (with AFP at HCC diagnosis and 6 months earlier). 2141 AFP det. in patients without HCC were collected (median: 6/patient; 1305 in SVR and 836 in VIR). ROC curves were used for the analysis of sen./esp., considering the first AFP det. in patients without HCC and the one at diagnosis in those with HCC.
Results: Most patients without HCC were male (65.3%), age 54 years, genotype 1 (79.3%) and CHILD-Pugh A (91.1%). In 83% SVR was obtained with DAA. The proportion of AFP det. >10, >15 y >20 ng/ml in SVR and VIR patients was 4.3% vs 45%, 0.45% vs 28.7% y 0.22% vs 21.1% (p <0.001). No SVR patient had det. >20 ng/ml and more than twice the previous det., while this fact was present in 2, 75% in VIR patients (p <0.001). Patients with HCC were mainly male (75.2%), genotype 1 (69.5%). Among them, 18 (17%) had developed HCC after SVR. The proportion of patients with AFp >10, >15 y >20 ng/ml at HCC diagnosis was 60, 9%, 59% y 49, 5% respectively, without differences between SVR and VIR patients (p >0.5). 28, 5% of patients had AFp >20ng/ml and more than twice the previous det. Area under the ROC curve of AFP for HCC diagnosis was 0, 77 (IC95%:0.71-0.83) in the global series, 0.65 (IC95%:0.57-0.73) in VIR patients and 0.75 (IC95%:0.59-0.92) in SVR. The value with higher diagnostic efficacy was 15 ng/ml in VIR patients (sen.58.6%; spe.73.4%) and 10 ng/ml in those SVR (sen.66.7%; spe.94.2%; VPP 48%, VPN 97.2%).
Conclusion: In HCV-cirrhosis, SVR leads to a normalization of AFP value, lowering the proportion of false positives. SVR also allows to reduce the value with most discriminative ability, increasing sensibility. As a
consequence, diagnostic performance of AFP substantially improves after SVR. These results open the door to re-evaluate AFP role in HCC screening in patients with HCV-cirrhosis.

LBP-05 Influence of treatment with direct-acting antivirals in hepatocellular carcinoma development in patients with Hepatitis C Virus-related cirrhosis

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Email: anagomezoutomuro@gmail.com

Background and aims: The influence of direct-acting antiviral (DAA) on hepatocellular carcinoma (HCC) development in patients with hepatitis C virus cirrhosis (HCV-C) remains controversial. Aim: to analyze the incidence of HCC after the start of DAA in patients with HCV-C compared with a historical cohort of untreated patients and variables associated with HCC development.
Method: 722 patients with HCV-C HCV-RNA + included in a surveillance program for the early diagnosis of HCC, biannual US and alphafetoprotein, were prospectively followed; 415 treated with DAA and 307 non-treated with DAA. The start of follow-up was the initial control in non-DAA and the start of treatment in DAA patients.
Results: 68% male, age 54 years (48-62), 87% Child A. Non-DAA patients were younger (p = 0.009) and had more advanced liver disease (lower albumine, prothrombin and platelets; p <0.05). There were no differences in Child (p = 0.99) or history of previous decompensation (p = 0.17). Most DAA patients (96%) reached sustained virological response. During a median follow-up of 36 months (18-42), 61 patients developed HCC (39 non-DAA and 22 DAA). In univariate analysis, HCC was associated with age>52 years (p = 0.005), HBsAg (p = 0.019), alcohol consumption (p = 0.008), AST>ULN (p = 0.015), BT>1 mg/dl (p <0.001), albumin <35 g/l (p <0.001), prothrombin ≤75% (p <0.001), platelet count <110x103/mm3 (p <0.001), Child B (p <0.001) and esophageal varices (p <0.001). The probability of developing HCC at 12, 24 and 36 months was 3.4%, 5.8% and 9.5% in DAA and 3.9%, 7.1% and 11.4% in non-DAA (p = 0.57). In multivariate analysis, variables independently associated with HCC were age>52 years (HR 3.16, 95% CI: 1.68-5.92), HBsAg (HR 3.87, IC95%: 1.19-12.59), alcohol consumption (HR 1.84; 95% CI: 1.04-3.26), AST>ULN (HR 4.37, 95% CI: 1.06-18.00), Child B (HR 2.53, IC95%: 1.35-4.76) and varices (HR 3.12, IC95%: 1.43- 6.77). DAA treatment was not associated with HCC development (HR 0.81, 95% CI: 0.46-1.41). Transplant-free survival at 12, 24 and 36 months was 99%, 93.7% and 89.7% in DAA and 93.4%, 86.8% and 78.4% in non-DAA (p <0.001).
Conclusion: Treatment with DAA in patients with HCV-C did not increase the risk of developing HCC. In this series no temporal relation between DAA start and HCC diagnosis was observed. The development of HCC was associated with advanced age and disease and presence of cofactors. Transplant-free survival was higher in DAA patients; it could explain the absence of reduction in the risk of developing HCC.

LBP-06 Identification of novel epigenetic targets in the fibrotic stroma associated with HCC.

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Background and aims: Liver fibrosis is an essential component of chronic liver disease (CLD) and hepatocarcinogenesis. Hepatic cirrhosis, the most advanced stage of CLD, is becoming a worldwide health problem, with high mortality rates and no effective antifibrotic therapies available. Several molecular mechanisms implicated in the activation of hepatic extracellular matrix (ECM) producing cells have been identified. These include epigenetic mechanisms involving changes in DNA and histone methylation regulating the expression of key genes. Therefore, the enzymes responsible for these epigenetic events, such as DNA and histone-methyltransferases, would be attractive therapeutic targets. We have recently demonstrated that histone methyltransferase G9a and DNA methyltransferase DNMT1, forming a gene expression regulatory complex, play a key role in hepatocarcinogenesis. We have developed first-in-class dual inhibitors of these enzymes with therapeutic potential and significant antitumoral effects. The present study evaluates G9a as a possible therapeutic target in hepatic fibrosis and examines the antifibrotic potential of CM-272, our lead compound dual inhibitor of G9a/DNMT1.

Results: G9a is overexpressed in the fibrotic liver and is induced during the activation process of hepatic stellate cells (HSCs) in culture. We observed that G9a plays a very important role in the response of these cells to TGFβ, the main pro-fibrogenic cytokine, as G9a knockdown impaired TGFβ-mediated signaling and gene regulation. Treatment with CM-272 significantly modified the gene expression profile induced by TGFβ in human HSCs, prevented their profibrogenic response and the metabolic adaptation of these cells to hypoxia. We observed a potent antifibrotic effect of CM-272 in *ex vivo* models of precision-cut liver slices obtained from rats and patients subjected to profibrogenic stimuli (PDGF+TGFβ). Finally, the antifibrotic effect of CM-272 was corroborated in mouse models of hepatic fibrosis, such as the CCl_4 administration and bile duct ligation, where a very significant antifibrotic effect of CM-272 was observed without signs of hepatic or systemic toxicity.

Conclusion: G9a plays an important role in the activation of HSCs. We have demonstrated the antifibrotic potential of a dual G9a/DNMT1 inhibitory molecule, tested in *ex vivo* and *in vivo* models without apparent toxic effects. Our data suggest a new strategy for the development of effective therapies against fibrosis.

LBP-07 Glycogen synthase 2 (GYS2) restricts tumor metastatic ability via attenuating autophagy and breaking glycogenic homeostasis in hepatocellular carcinoma

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Email:

Background and aims: Aberrant metabolic reprogramming tightly linked to carcinogenesis or tumor progression. However, rare attention has been paid to glycogen metabolism. The regulation of glycogen in liver, as the main organ of glycogen metabolism, might be different to other organs with unknown mechanisms. Here we are willing to demonstrate that Glycogen synthase 2 (GYS2) restrict tumor metastatic ability via attenuating autophagy and breaking glycogenic homeostasis in hepatocellular carcinoma.

Method: GYS2 expression pattern was investigated by both weighted gene co-expression network analysis and Robust Rank Aggregation method and verified by RT-qPCR, westernblot and immunochemistry in HCC tissue. MTS, metastasis mouse model, transwell assay and tandem mCherry-GFP-LC3 fusion protein assay were utilized to assess the function of GYS2 on proliferation, metastasis and autophagy of HCC cells in vitro and in vivo.

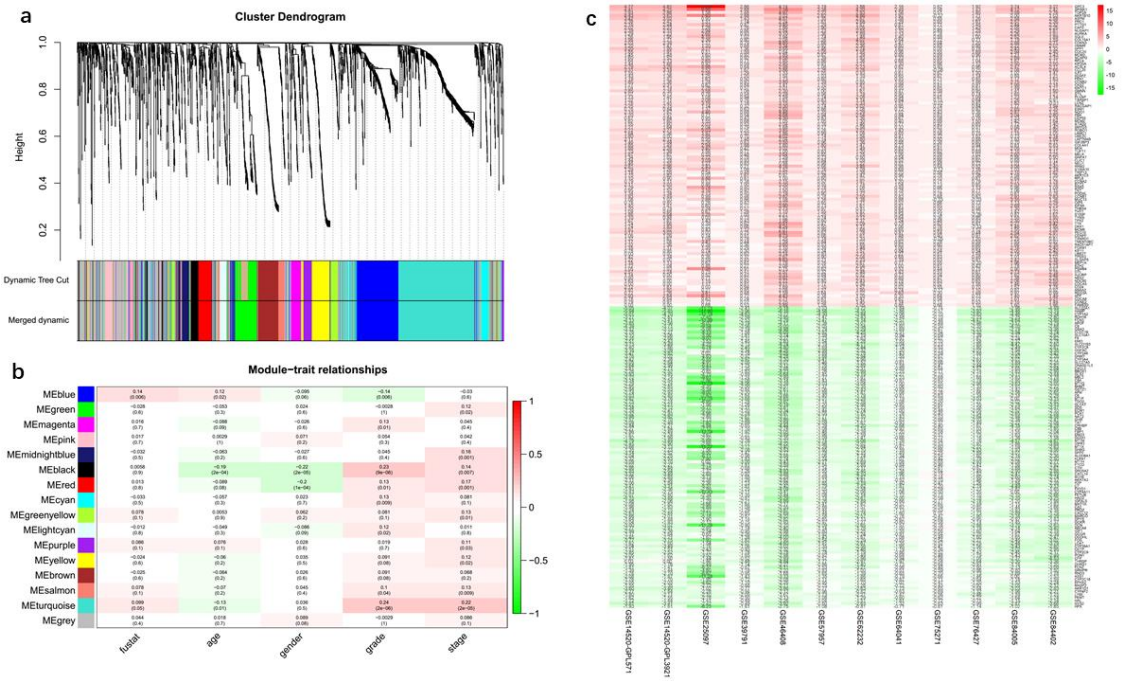
Results:

GYS2 downregulation was quite prevalent in HCC tissues and closely correlated with worse overall survival .Furthermore, GYS2 overexpression significantly suppressed cell growth, migration and autophagy, the expression of ZEB1, Snail, βcatenin, and increased E-cadherin level. Overexpression of GYS2 induced a decline of PYGL even in starvation condition, consequent glycogen accumulation led to increased reactive oxygen species (ROS) levels and celluar senescence.

Conclusion: In summary, our findings suggested that GYS2 serves as a prognostic factor and functions as a tumor suppressor by inhibiting autophagy and interpreting glycogenic homeostasis in HCC. GYS2 might be a promising target for prevention, treatment and prognostic prediction of HCC.

Figure:

Figure1



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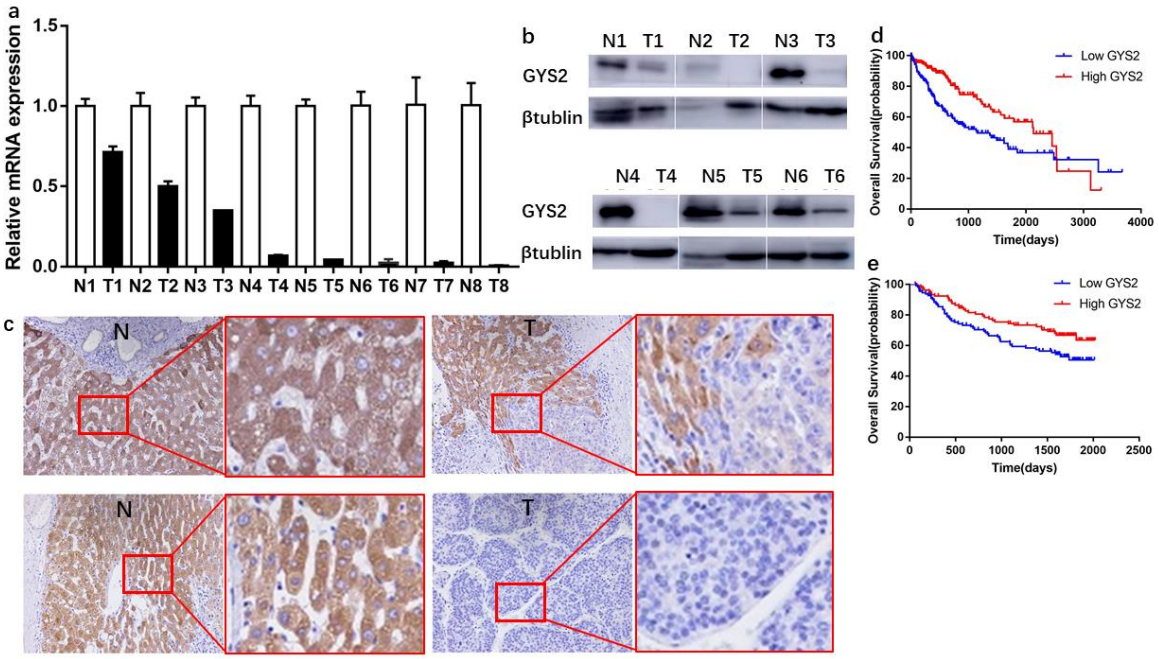

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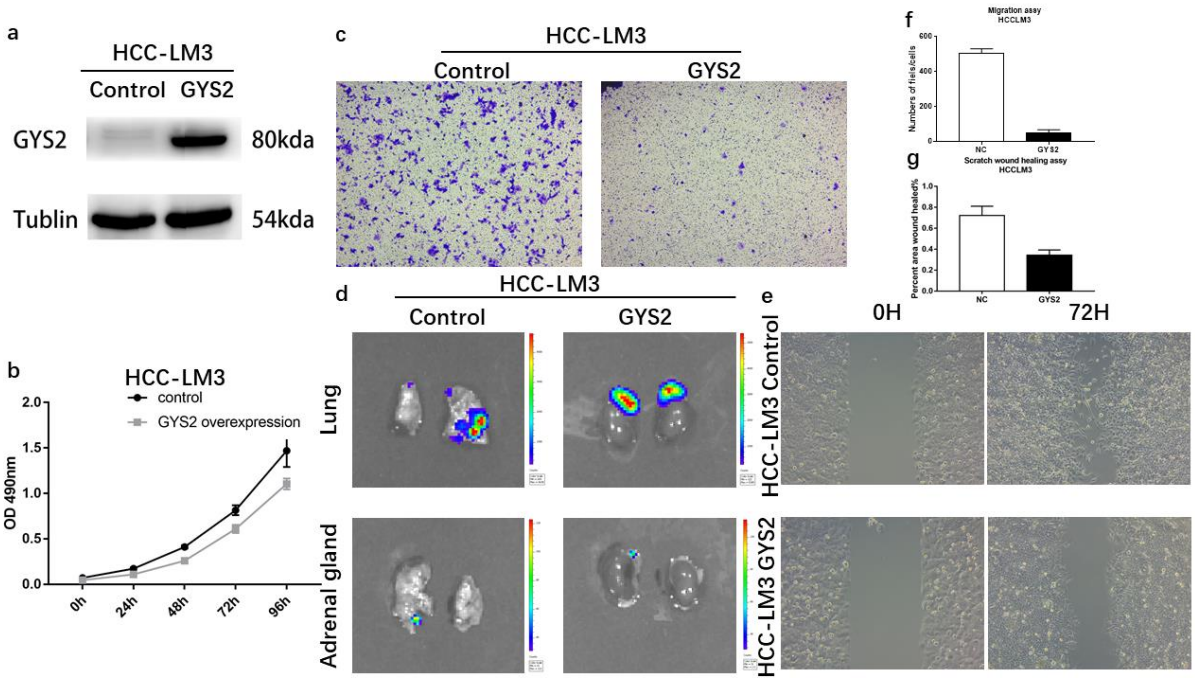

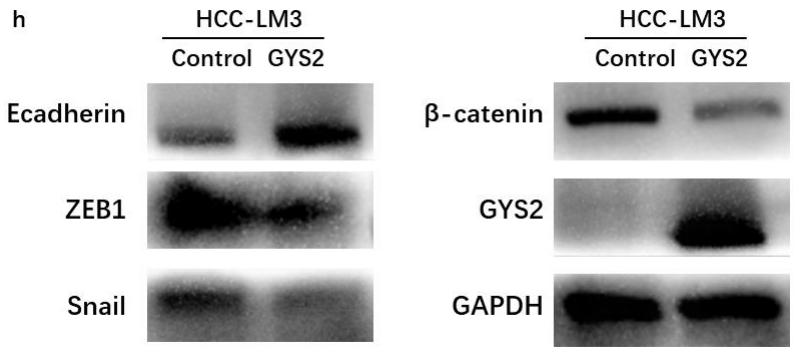

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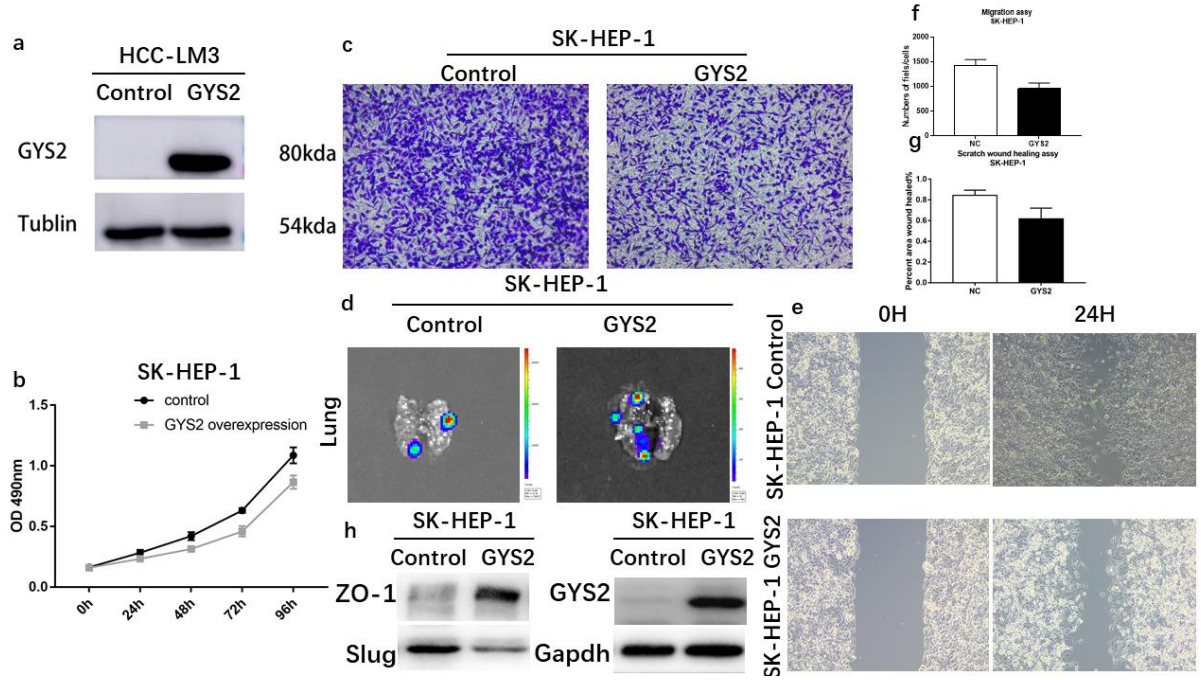

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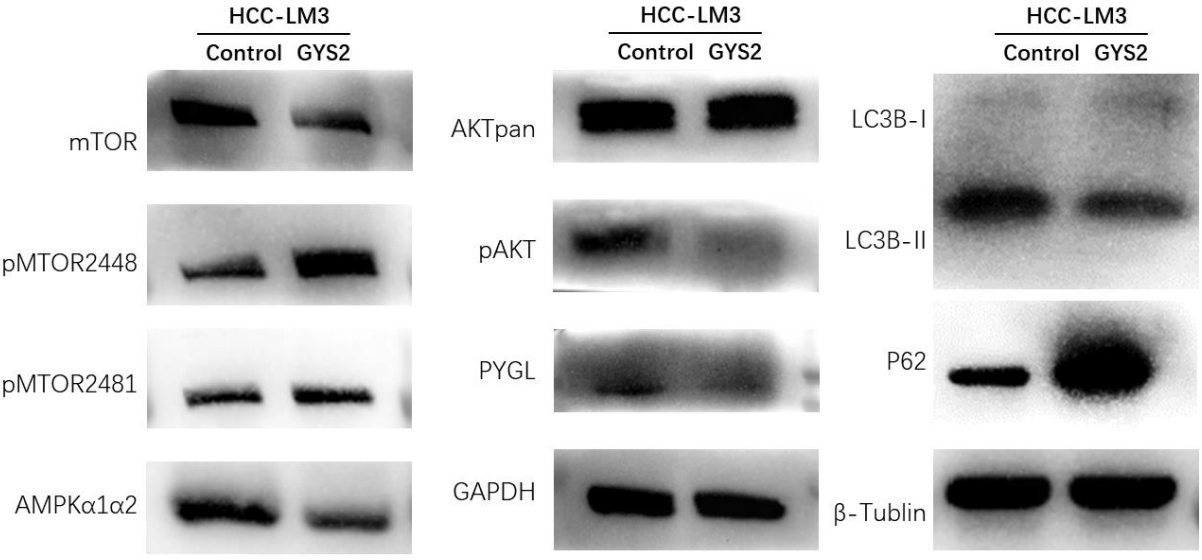

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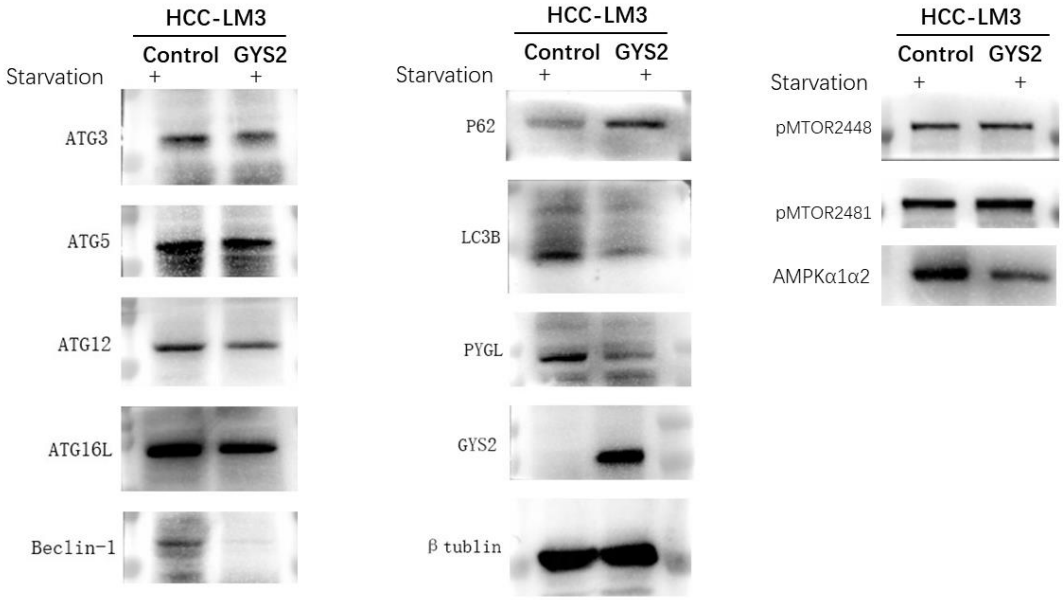
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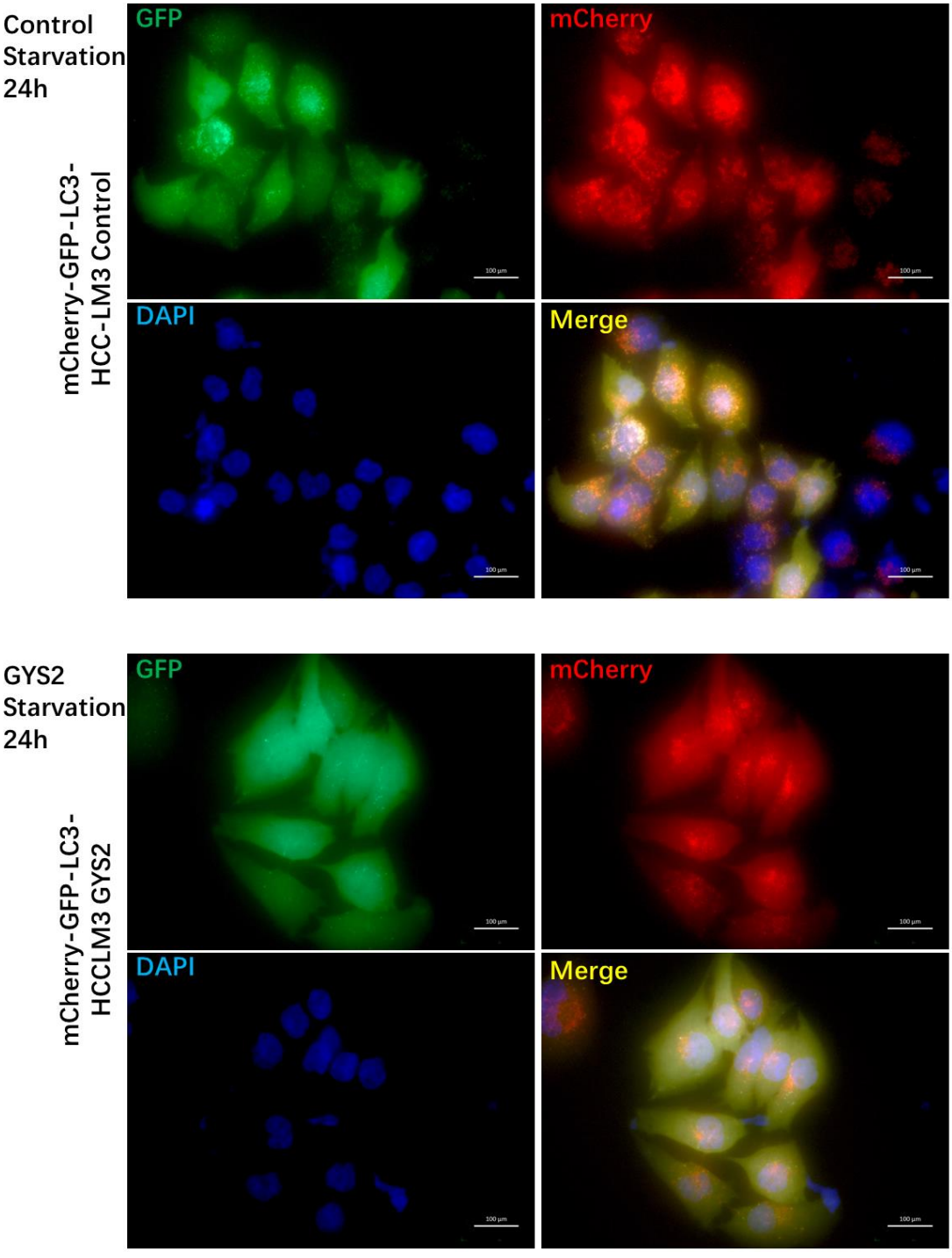
Figure7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GFP
mCherry
DAPI
Merge

LBP-08 The EGF-HER2/ErBb2-JNK axis drives intrahepatic cholangiocarcinoma (CCA)

development

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Background and aims: Intrahepatic cholangiocarcinoma (CCA) is a malignant epithelial neoplasm derived from primary and secondary bile tracts, and accounts for 5-10% primary liver cancer whose incidence and mortality are steadily increasing. Unfortunately, the pathophysiology underlying CCA development is still very limited and, thus, it is urgent to unveil the mechanisms of CCA progression in order to develop effectively therapy strategies. The c-Jun N-terminal kinase (JNK) signaling pathway is pivotal in regulating cell differentiation and thus we aimed to investigate the role of JNK signaling in CCA development.
Method: JNK signaling in hepatocytes was inhibited by crossing C57BL/6 *AlbCre* with *Jnk1/Jnk2*$^{LoxP/LoxP}$ (*Jnk*$^{\Delta hepa}$) mice. *Jnk*$^{\Delta hepa}$ mice were interbred with hepatocyte-specific *Nemo*-knockout mice (*Nemo*$^{\Delta hepa}$), a model of chronic liver inflammation and spontaneous hepatocarcinogenesis (HCC), to generate *Nemo*$^{\Delta hepa}$/*Jnk*$^{\Delta hepa}$ mice. In parallel, mice *Jnk1*$^{\Delta hepa}$/*Jnk2*$^{-/-}$ mice were challenged with dinitroethylsamine (DEN, 25 mg/kg) and carbon tetrachloride (CCl_4, 0.5 ml/kg) for 4 months. The phenotype was characterized in both models and active signaling pathways were blocked using specific inhibitors.
Results: Compound deletion of Jnk1 and Jnk2 in hepatocytes mitigated HCC but, in contrast, triggered CCA development in *Nemo*$^{\Delta hepa}$ mice as demonstrated with significant higher levels of ALT, AST, AP and GLDH. Interestingly, microscopic analysis of liver sections showed strong-ductular proliferation and cyst formation in *Nemo*$^{\Delta hepa}$/*Jnk*$^{\Delta hepa}$ livers. Indeed, JNK deficiency in hepatocytes of *Nemo*$^{\Delta hepa}$ (*Nemo*$^{\Delta hepa}$/*Jnk*$^{\Delta hepa}$) caused elevated fibrosis, increased apoptosis, increased compensatory proliferation, and elevated inflammatory cytokines expression. Next, we sought to investigate the impact of molecular pathways in response to compound JNK deficiency in *Nemo*$^{\Delta hepa}$ mice. We found *Nemo*$^{\Delta hepa}$/*Jnk*$^{\Delta hepa}$ livers exhibited overexpression of the IL-6/Stat3 pathway in addition to EGFR-Raf-MEK-ERK cascade. To test the functional relevance of this finding we administered Lapatinib-a dual tyrosine kinase inhibitor interrupting ErbB2 and EGFR signaling-to *Nemo*$^{\Delta hepa}$/*Jnk*$^{\Delta hepa}$ mice. Lapatinib effectively inhibited cyst formation, improved transaminases and effectively blocked EGFR-Raf-MEK-ERK signaling.
Conclusion: The JNK signaling pathway plays an essential role in hepatoblast differentiation and CCA formation via activation of the EGFR-Raf-MEK-ERK pathway. Lapatinib therapy successfully prevented CCA development via inhibition of EGFR/ErbB2 phosphorylation, opening new therapeutic avenues for the treatment of this biliary tract cancer.

ePOSTER ABSTRACT PRESENTATIONS

OP-01 Genome wide RNA expression analysis identifies CD44 positive macrophages as promoters of hepatocyte proliferation and the development of NAFLD-HCC

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Background and aims: NAFLD associated hepatocellular carcinoma (HCC) is steadily increasing, with cirrhosis often absent in affected individuals. We have previously reported that C3H/HeH mice fed the American lifestyle (ALIOS) diet develop more HCC than matched diet controls, in association with steatosis and lipogranulomas, rather than with other features of NASH-namely ballooning degeneration and lobular inflammation. The underlying mechanisms remain poorly understood.
Method: 72 mice were fed ALIOS or control diets from age 8 weeks until culling at 48 weeks. In 24, the diet was supplemented with the antioxidant, bucillamine (20mg/kg) for the final 24 weeks. Hepatic DNA damage and proliferation were assessed with H2AX and Ki67 immunohistochemistry (IHC). RNA-sequencing was performed on non-tumour liver, with transcriptomics analysed by unsupervised clustering. Differentially expressed (DE) genes between clusters were identified using 'R'. Top pathways and networks enriched by DE genes were analysed using Gene Set Enrichment (GSEA) and Ingenuity Pathway Analysis (IPA).
Results: In ALIOS fed mice, bucillamine treatment eradicated ballooning degeneration and dramatically reduced NAS scores and DNA damage, but had no impact on HCC burden. Bucillamine treatment also had no impact on steatosis, lipogranulomas or Ki67 scores, all of which remained highly significantly associated with the development of HCC (KI67 positive nuclei were higher in the ALIOS fed mice ($p < 0.001$) and strongly associated with tumour development (Spearman rho 0.506, $p = 0.012$). Unsupervised clustering of the non-tumour liver RNA-seq data distinguished two groups (G1 and G2), with G2 consisting entirely of ALIOS fed mice developing HCC. 248 genes were differentially expressed between G1 and G2. GSEA of the DE gene list ranked Macrophage enriched metabolic network (MEMN) as the top enriched pathway (72/248, q-value 3.16E-53). IPA highlighted increased cell survival and proliferation genes in G2, with the adhesion receptor *CD44* identified as the top DE upstream regulator (Z-score = 3.351, overlap p value = 1.47E-15). By IHC, CD44 was exclusively expressed in hepatic macrophages and a 6-fold increase in CD44 positive macrophages-often clustered around free lipid droplets- was observed in ALIOS vs control fed mice ($p < 0.0001$). Increased CD44 positive macrophages (much more so than CD68 or F480 positive macrophages) was strongly associated with liver weight, the number of Ki67 positive nuclei and the presence of lipogranulomas (Spearman rho 0.643, $p = 0.001$, 0.855, $p < 0.0001$, 0.734, $p < 0.0001$ respectively)
Conclusion: In the ALIOS C3H/HeH mouse model, hepatocyte proliferation and HCC development was strongly associated with the presence of CD44 positive macrophages in the liver microenvironment, identifying these cells as candidate drivers of NAFLD-HCC. Studies in human NAFLD are ongoing and will be presented.

OP-02YI NASH-related liver carcinogenesis is critically affected by hypoxia-inducible factor 2-alpha

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Background and aims: Hypoxia and hypoxia inducible factors (HIFs) are believed to significantly affect the progression of chronic liver diseases (CLD). Recently, we showed that hepatocyte HIF-2α activation is a key feature in both human and experimental NAFLD and significantly contributes to disease progression in both experimental animals and human patients. In the present study we investigated the contribution of hepatocyte HIF-2α in promoting the development of NAFLD/NASH-associated hepatocellular carcinoma (HCC).
Method: The role of HIF-2αwas investigated: a) in human HCC liver specimens from NAFLD/NASH patients, b) in mice carrying hepatocyte-specific deletion of HIF-2α (HIF-2α fl/fl/Alb-Cre mice) receiving diethyl-nitrosamine (DEN) administration plus choline-deficient L-amino acid refined (CDAA) diet (DEN/CDAA protocol); c) in HepG2 stably transfected to overexpress HIF-2α vs cells transfected with the empty vector.
Results: HIF-2α, as detected by immunohistochemistry (IHC), was expressed in HCC specimens from NAFLD/NASH patients, with higher expression in patients experiencing early tumour recurrence. Following the treatment with the DEN/CDAA protocol, mice carrying hepatocyte specific deletion of HIF-2α showed a significant decrease in the volume and number of neoplastic liver tumour masses in transgenic mice vs control littermates. Liver tumours in HIF-2α transgenic mice were also characterized by a significant decrease in: a) transcripts levels of tumour associated macrophages and fibroblasts/myofibroblasts markers (including F4/80 and α-smooth muscle actin); b) transcript levels for critical and HIF2α-related target genes, including EPO, c-Myc and CXCR4. In vitro data indicate that HIF-2α is also able to significantly induce cell proliferation, a process that is impaired also in the HIF-2α fl/fl/Alb-Cre mice compared to the control littermates.
Conclusion: These results indicate that theactivation of HIF-2α in hepatocytes has a critical role in the development of experimental liver carcinogenesis in a dietary NAFLD/NASH-related environment.

OP-03YI Mixed HCC-ICC Liver Cancer Derived From Hepatic Progenitor Cells- a Lineage Tracing Investigation in a Mouse Liver Inflammation Model

Nofar Rosenberg[1], Matthias Van Haele[2], Neta Barashi[1], Klaus Kaestner[3], Mathias Heikenwälder[4], tania roskams[2], Hilla Giladi[1], Eithan Galun[1]
[1]The Hebrew University, Goldyne savad institute of gene therapy, Jerusalem, Israel, *[2]University of Leuven, Department of translational cell and tissue Research, Leuven, Belgium*, *[3]University of Pennsylvania Perelman School of Medicine, Department of Genetics and Center for Molecular Studies in Digestive and Liver diseases, Philadelphia, United States*, *[4]German Cancer Research Centre, Chronic Inflammation and Cancer, Heidelberg, Germany*
Email: nofar.ros@gmail.com

Background and aims: Primary liver cancer is the second leading cause of cancer-related death worldwide. Primary liver cancers include: Hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC) and Mixed HCC-ICC tumor. Preceding the development of primary liver cancer, there is usually cirrhosis which results from a prolonged period of chronic inflammation. It has been proposed that hepatic progenitor cells (HPCs) could contribute to hepatocarcinogenesis. However, this has not yet been proven. These cells proliferate in response to injury and chronic inflammation in the liver. In this study, we aimed to determine whether HPCs contribute to liver cancer development in the MDR2 KO mouse model of inflammation-induced HCC.
Method: In order to enable tracing of progenitor cells, we generated a transgenic mouse based on the MDR2 KO that harbours a YFP reporter gene driven by the Foxl1 promoter, the promoter of a liver progenitor specific marker. These mice (MDR2 KO$^{Foxl1CRE;\ RosaYFP}$) develop chronic inflammation by the age of 1 month and HCCs by the age of 16 months followed by Mixed HCC-ICC tumors at the age of 18 months.
Results: At the age of 3 months, upon severe inflammation, YFP positive HPCs proliferate and differentiate, giving rise to both cholangiocytes and hepatocytes. In addition, at the age of a year and later, HPCs are present in the chronically inflamed livers and within dysplastic nodules (DN). Within the livers of 16-month old MDR2 KO$^{Foxl1CRE\ ;RosaYFP}$ only a minority of DNs were positive for YFP expression. Furthermore, the HCC tumors that have developed, were YFP negative, but contained scattered YFP-positive HPCs. At later stages (18-month) these mice developed Mixed HCC-ICC tumors and very few ICC. Interestingly, the Mixed tumors were YFP positive, implying that they were derived from HPCs. These HCC-ICC YFP positive tumors accounted for 45% of the total tumors observed and they also express stem cells markers including Epcam, Cd24a, Cd133 and Krt19. These findings recapitulate the characteristics of human Mixed ICC-HCC tumors which also shown to have stem cell-like features. Next generation sequencing (NGS) analysis of human and mouse Mixed ICC-HCC tumors, revealed the presence of HPCs gene signature, implying that they originate from Foxl1 HPCs cells. In addition, RNA seq analysis revealed that both, human and mouse Mixed HCC-ICC tumors have a very similar pro-survival pathway signature. Most significantly, IL6 signalling was found to be up-regulated in these Mixed tumors at the RNA and protein levels.
Conclusion: Taken together, our results suggest that mixed HCC-ICC but not HCC tumors, originate from HPCs in the inflammation induced liver cancer model, and that the driver of this process involves the IL6 signalling pathway.

OP-04YI European Cholangiocarcinoma (EU-CCA) Registry: An Initiative to Broaden Awareness on the Second Most Common Primary Liver Cancer

Alvaro Santos-Laso[1], Angela Lamarca[2], Adelaida La Casta[1], Chiara Braconi[3 4], Jorge Adeva[5], Matthias Evert[6], Alejandro Forner[7], Rocio IR Macias[8], Marek Krawczyk[9], Laura Izquierdo-Sánchez[1], Vincenzo Cardinale[10], Guido Carpino[11], Domenico Alvaro[12], Juan Valle[2], Jesús María Banales[1]

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Email: jesus.banales@biodonostia.org

Background and aims: Cholangiocarcinomas (CCAs) are classified into intrahepatic (iCCA), perihilar (pCCA) or distal (dCCA). Their etiologies are mostly unknown and their pathogenesis is poorly understood. Large international registries of CCA patients with demographic, biochemical, clinical and histopathological information are missing and necessary to better understand this disease and improve its management.

Method: The European CCA Registry is an international multicenter initiative supported by EASL (Registry Award 2016) that includes patients with CCA from 8 institutions and 4 countries in the secure online platform REDCap. Clinical data was retrospectively (2010) and prospectively (2016) collected by experts in the field.

Results: A total of 1, 011 records are entered, including 48% iCCAs, 23% pCCAs, 28% dCCAs and 1% CCA-HCC mixed tumors. Mean age at diagnosis was 65.6, and the male/female ratio 1:0.76. The most common known risk factors were cirrhosis (6.4%), viral hepatitis (3.1% HCV; 1.8% HBV), diabetes (20.5%), obesity (15.2%), alcohol (17.2%) and presence of primary sclerosing cholangitis (PSC: 1.3%). Serum levels (mean; IU/L) of biochemical markers of cholestasis (GGT: 417; ALP: 313) and liver injury (ALT: 74.1; AST: 60.8), as well as non-specific tumor markers (CA19.9: 5, 138 UI/ml; CEA: 163 ng/ml) were altered. These biomarkers were considered for diagnosis together with imaging (53.1%: MRI, CT, USG, ERPC) and pathological (34.5%: biopsy/cytology) approaches. Tumor staging (TNM; AJCC 7th Edition) at diagnosis revealed that 39.7% of cases had stages I-III, 39.9% stage IV, and 20.4% unknown. Moreover, 38.9% of patients underwent surgical resection (R0-R1) (81.0% I-III; 12.2% IV; 6.8% unknown) with an overall survival (OS) of 18 months (IC95: 16-22). On the other hand, 61.1% of the tumors were unresectable: 70% (16.8% I-III; 59.7% IV; 23.5% unknown) received chemo- or locoregional therapies, and 30% did not receive treatment (10.2% I-III; 57.2% IV; 32.5% unknown). Chemo- or locoregional therapies provided an OS of 10 months (IC95: 9-11), whereas non-treated patients exhibited an OS of 4 months (IC95: 3-6).

Conclusion: This international initiative shows the current management of patients with CCA, and demonstrates the need of international collaborations to improve diagnosis, staging and treatment. The European CCA Registry, which also includes biological samples, emerges as a unique and extraordinary platform for future collaborative studies.

OP-05YI Combined CXCR2 inhibition and Anti-PD1 therapy alters neutrophil and T cell infiltration and limits HCC progression.

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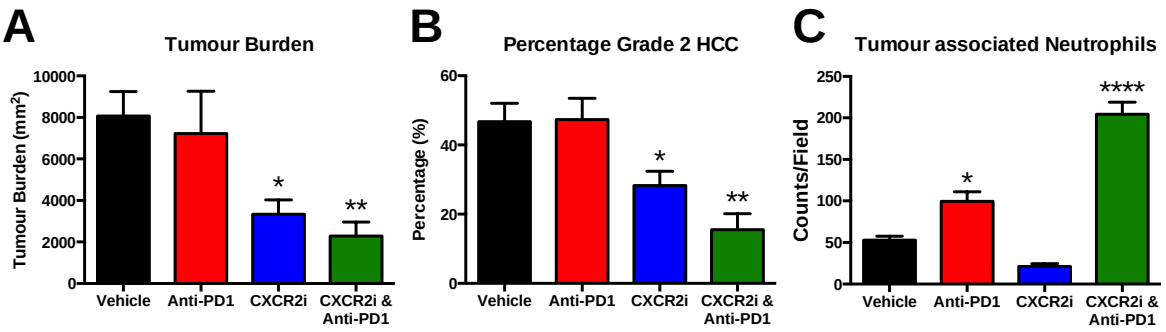
Background and aims: Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and is a leading cause of cancer-related death worldwide. Immune cells play a complex role in cancer, both promoting and limiting tumour progression. In the tumour microenvironment immunosuppressive neutrophils limit T cell activation, promoting tumorigenesis. Using a model of NAFLD associated HCC, we explored the therapeutic impact of impairing neutrophil chemotaxis into the liver, while promoting T cell activation.
Method: The mouse DEN/ALIOS model of NAFLD-HCC was used. Briefly, male C57Bl6/j mice receiving a single intraperitoneal injection of N-Nitrosodiethylamine (DEN) at 2 weeks old were placed on to American Lifestyle-Induced Obesity Syndrome (ALIOS) diet at 8 weeks until culling at 40 weeks. Mice received 'late stage' vehicle control, a CXCR2 inhibitor (gavage twice daily) and/or an anti-PD1 antibody (Biolegend #114108 200μg twice weekly by ip injection) for the final 12 weeks after tumours have already been established.
Results: Late stage treatment with anti-PD1 therapy in the DEN/ALIOS model had no effect on tumour stage or grade at 40 weeks. Treatment with the CXCR2 inhibitor limited both tumour stage and grade, with the combination therapy reducing them further **(Figure A-B)**.
Late stage treatment also had a profound effect on hepatic neutrophil number. Anti-PD1 therapy increased neutrophil infiltration compared to vehicle control mice, whereas CXCR2 inhibition significantly reduced it. Interestingly, combined therapy resulted in a large influx of neutrophils into both tumour and non-tumour liver **(Figure C)**. We hypothesise that the combination therapy preferentially promotes the influx of anti-tumour rather than pro-tumour/immunosuppressive neutrophils.
Anti-PD1 therapy resulted in significant increases in the number of both CD8 and CD4 positive T cells in the tumour and non-tumour. CXCR2 inhibition was associated with elevated CD4 positive T cells only, while the combination restored associations with both CD8 and CD4 positive T cells, with both being significantly elevated compared to vehicle controls.
The effects of CXCR2 inhibition and anti-PD1 therapy alone compared to their combination highlights critical tumour promoting bidirectional crosstalk between neutrophils and T cells in the tumour microenvironment.
Conclusion: Our data provides the first evidence, in a murine model of late stage NAFLD-HCC, that dual targeting of neutrophils and T cells with the combination of a CXCR2 inhibitor and anti-PD1 therapy, combats HCC progression and has therapeutic potential.
Figure:



OP-06 Non-autonomous induction of endothelial Inducible T-cell co-stimulator ligand may underpin immune-mediated senescence surveillance

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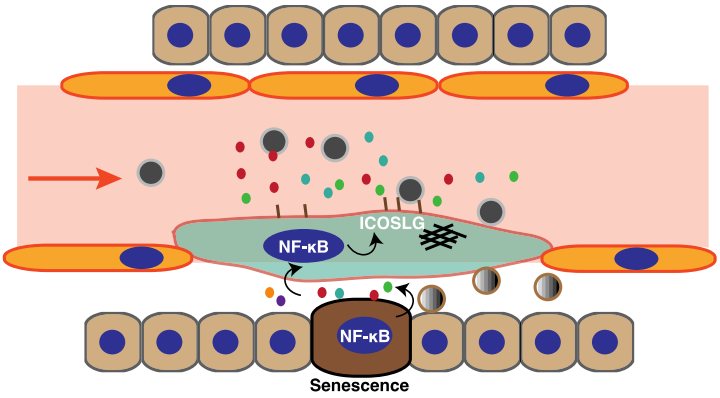
Background and aims: Oncogene-induced senescence (OIS), a persistent form of cell-cycle arrest and tumour suppressor mechanism, has profound effects on the tumour microenvironment (TME) through the senescence-associated secretory phenotype (SASP). The SASP drives T-lymphocyte dependent clearance of senescent cells; failure of this senescence surveillance leads to tumorigenesis. We hypothesise that OIS cells will modulate endothelial behaviour and thereby control immune cell recruitment and behaviour in the TME.
Method: To study OIS to endothelial signalling *in vitro* we use human diploid fibroblasts containing an inducible ER:HRAS^{G12V} construct to generate OIS-conditioned media and human liver sinusoidal endothelial cells (LSECs) or HUVECs as target cells. To study OIS to endothelial signalling *in vivo* we utilise hydrodynamic tail-vein (HDTV) injected NRAS^{G12V}-containing transposons generating murine hepatocyte OIS, before analysis of LSEC behaviour.
Results: Using transcriptomics in LSECs incubated in control or OIS-conditioned media, we find SASP-induced activation of endothelial NF-kB signalling, with upregulation of cytokines, chemokines and the immune co-stimulatory ligand *ICOSLG*. Inhibition of NF-kB prevents the OIS-dependent increase in both gene expression and lymphocyte adherence in the endothelium. We validated OIS-induced NF-kB-dependent upregulation of *ICOSLG* in multiple endothelial cell types. In mice, hepatocyte OIS drives the upregulation of multiple NF-kB-target genes in LSECs, including *Cxcl1* and *Icosl*, demonstrating OIS hepatocyte to endothelial cell signalling *in vivo.* OIS hepatocytes demonstrate autonomous upregulation of chemokines, but not *Icosl*. Use of Icosl-blocking antibodies after induction of hepatocyte OIS completely abrogates the time-dependent immune-mediated senescence surveillance. Mass-cytometry based intrahepatic deep immunophenotyping shows that OIS hepatocytes drive an intrahepatic enrichment of activated Icos+CD4+ T-lymphocytes and CD11b+Ly6G+ granulocytes, that is lost with Icosl blockade, suggesting that induced endothelial behaviours might control immunocyte recruitment to the TME.
Conclusion: In conclusion, we propose that OIS hepatocytes drive non-autonomous induction of NFkB-dependent *Icosl* expression in endothelial cells. Induced endothelial *Icosl* has profound effects upon immune-cell recruitment and may control immune-mediated senescence surveillance.
Figure:



OP-07 Ramucirumab for patients with hepatocellular carcinoma and elevated alpha-fetoprotein following sorafenib treatment: exploratory analysis of REACH-2 trial results by albumin-bilirubin grade and Child-Pugh score

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Background and aims: Patients (pts) with advanced hepatocellular carcinoma (HCC) and elevated alpha-fetoprotein (AFP) have a poorer prognosis compared to the general HCC population. As reported, REACH-2 (NCT02435433) met its primary end point; ramucirumab (RAM) (anti-VEGFR2 monoclonal antibody) improved overall survival (OS; HR 0.710, p = 0.0199) and progression-free survival (PFS; HR 0.452, p <0.0001) compared to placebo (PL) in pts with HCC and elevated AFP without significant toxicity or compromise in patient-reported outcomes. Albumin-bilirubin (ALBI) grade has been proposed as an alternative approach to the Child-Pugh (CP) score to assess liver function and is based solely on serum bilirubin and albumin levels.
Method: REACH-2 was a randomized (2:1), double blind, phase 3 study of RAM (8 mg/kg IV Q2W) vs PL in pts with advanced HCC and elevated AFP (≥400ng/ml) who progressed on or were intolerant to sorafenib. Here we present exploratory analyses of the ITT population (n = 292) by ALBI grade (1 or 2) and CP score (5 or 6). OS was evaluated by the Kaplan-Meier method and HR were estimated using Cox models.
Results: Within each group defined by ALBI grade (ALBI-1, n = 143; ALBI-2, n = 144; missing grade, n = 5) and CP score (CP-5, n = 177; CP-6, n = 115), baseline characteristics between treatment arms were similar. In general, ALBI-1 or CP-5 were both associated with ECOG PS 0, less macrovascular invasion, and a higher proportion of hepatitis B, compared to ALBI-2 or CP-6. OS curves (pooled treatment arms) by ALBI-1 (10.8 mo) or CP-5 (10.6 mo), and ALBI-2 (5.3 mo) or CP-6 (5.9 mo) resembled each other. The prognostic values are similar, OS HR (ALBI-1 vs AlBI-2) = 0.534 and HR (CP-5 vs CP-6) = 0.549. PFS and OS by scoring system are shown in Figure below. The pattern of treatment-emergent adverse events (TEAEs) between arms was comparable to the ITT population. ALBI-2 or CP-6 were both associated with a higher proportion of Grade ≥3 TEAEs, compared to ALBI-1 or CP-5.
Conclusion: Both ALBI and CP scoring systems appear to have similar prognostic utility when applied to the REACH-2 population.

Figure:

Unstratified HR (95% CI)	CP-5 (n = 177)	CP-6 (n = 115)	ALBI-1 (n = 143)	ALBI-2 (n = 144)
OS [RAM vs PL]	0.70 (0.48, 1.02)	0.82 (0.53, 1.26)	0.64 (0.42, 0.96)	0.93 (0.63, 1.38)
PFS [RAM vs PL]	0.43 (0.30, 0.61	0.56 (0.37, 0.86)	0.37 (0.25, 0.55)	0.61 (0.41, 0.90)

OP-08YI A multicenter international study of sorafenib treatment in patients with hepatocellular carcinoma and chronic kidney disease undergoing hemodialysis

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Background and aims: As patients with hepatocellular carcinoma (HCC) under dialysis are excluded from clinical trials, there are no data about safety and efficacy of sorafenib in this specific population, which has no formal contraindication for this treatment. The aim of this study is to evaluate the rate, prevalence, tolerability and effectiveness of HCC patients undergoing dialysis and being simultaneously treated with sorafenib (dialysis-sor) in clinical practice.
Method: Retrospective, international, multicenter study, including centers from Latin-America and Europe to evaluate the aim of the study. For dialysis-sor patients, baseline characteristics as well as dose modifications, adverse events, treatment duration, and overall survival were recorded.
Results: Of 6156 HCC patients treated with sorafenib between June/2006 and March/2018 in 44 centers, 22 were dialysis-sor patients. Median age of the 22 dyalisis-sor was 65.5 years, 83.4% were male, 40.9% had hepatitis C, 75% had Child-Pugh A, 85% were BCLC C, and 54.6% had ECOG-PS 1. 17/22 patients presented history of arterial hypertension (AHT), 14/22 had diabetes mellitus (DM) and 3/22 had heart disease. 12/22 patients presented with both AHT and DM, which also were the leading causes for chronic kidney disease. 15/22 patients started sorafenib at full dose, while 7/22 at half dose. 17/22 of patients required at least one dose modification. Median time to first dose modification was 2.4 months (IQR 0.8-3.8). 5 patients never required any dose modification. Maximum and medium number of dose modifications were 4 and 2, respectively. The most frequent causes of first dose modification were asthenia 3/22, worsening of ECOG-PS 3/22 and diarrhea 3/22.
Median treatment duration was 10.8 months (IQR 4.5-16.9) and median overall survival was 17.5 months (CI 95%; 7.2-24.5). 18/22 patients permanently discontinued sorafenib and the remaining are still on treatment. The causes of sorafenib discontinuation were tumor progression 14/18, sorafenib-related 2/18 (diarrhea and peripheral arterial thrombosis) and non-sorafenib related AE 2/18 (liver decompensation and pulmonary edema).
Conclusion: This is the largest cohort describing patients with HCC under hemodialysis who received sorafenib treatment. Our data show that median time to the first dose modification, treatment duration, and overall survival were comparable to the results of the non-dialysis population, obtained from large clinical trials and real-world cohorts. However, in these patients, asthenia appears to be the main treatment related adverse event.

P01-01YI The dual role of RIP3-dependent signalling in controlling steatosis and carcinogenesis in experimental non-alcoholic steatohepatitis

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Background and aims: Regulated necrosis or necroptosis was recently described as a novel cell death pathway activated downstream of death receptor stimulation and dependent on receptor-interacting protein 3 (RIP3) kinase activity. Although necroptosis has already been implicated in the pathogenesis of non-alcoholic fatty liver disease (NAFLD), its specific contribution remains poorly explored. Here, we aimed to evaluate the impact of RIP3 signalling in steatosis, inflammation, fibrosis and carcinogenesis associated with experimental NAFLD.
Method: C57BL/6 wild-type (WT) or RIP3-deficient ($RIP3^{-/-}$) 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. Tissue samples were processed for histological and biochemical analysis of hepatic damage and carcinogenesis, insulin resistance and oxidative stress, and for lipidomic analysis.
Results: CDAA-fed WT mice exhibited all the typical histological features of liver injury associated with non-alcoholic steatohepatitis, including steatosis, hepatocellular ballooning, immune cell infiltration, and fibrosis, which became more prominent over time. RIP3 deficiency ameliorated CDAA-induced inflammation and fibrosis, and decreased the NAFLD activity score. In agreement, hepatic gene expression of pro-inflammatory mediators was also significantly decreased in CDAA-fed $RIP3^{-/-}$ mice, compared with WT, at both 32 and 66 weeks. Intriguingly, $RIP3^{-/-}$ mice displayed increased body weight gains, as well as increased liver fat accumulation at both time-points, compared with WT mice on the CSAA or CDAA diets. Lipidomic analysis showed that deletion of RIP3 shifted hepatic lipid species profiles, while increasing insulin resistance, as assessed by homeostasis model assessment-estimated insulin resistance, compared with WT mice on CSAA or CDAA diet. Concomitantly, insulin receptor phosphorylation in muscle tissue was decreased at 66 weeks. Finally, $RIP3^{-/-}$ mice on the CDAA diet for 66 weeks tended to display reduced incidence of macroscopic preneoplasic nodules, accompanied by significantly reduced Ki67 positive hepatocytes. Indeed, microarray analysis and subsequent validation studies showed that the absence of RIP3 hampered the expression of oncogenes and signalling pathways controlling tumour microenvironment.
Conclusion: Overall, hepatic RIP3 plays an opposing role in controlling steatosis versus inflammation and carcinogenesis in CDAA-fed mice, leading to dissociation between these phenomena that are usually considered linked in NAFLD. Funding: PTDC/BIM-MEC/0895/2014; SAICTPAC/0019/2015 from FCT, Portugal.

P01-02YI Liver NK cells from NLG4 KO mice inhibited progressions of hepatocellular carcinoma of C57BL/6 mice model through decrease in p53 and AKT expressions

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Background and aims: Cirrhosis is a main risk factor for hepatocellular-carcinoma (HCC) development. However, direct role of NK cell in HCC-progression is not fully understood. Therefore, we aimed to study the role of neuroligin-4 (NLG4) receptor of NK cells in models of HCC in C57BL/6 mice.
Method: HCC cell-line (Hep3B) secreted high levels of alpha-feto-protein (αFP) were injected in the back of irradiated C57BL/6 (sub-lethal dose) and liver NK cells from both WT and homozygous NLG4$^{-/-}$ (KO) mice were transplanted at day 5 following HCC injections. Hepatic tumor sizes and serum αFP were then assessed from day 6 till day 14. Liver P53 and AKT expressions by RT were tested at day of sacrifice.
Results: Tumor mass increase in animals with HCC injections and was associated with elevated αFP serum levels in all tested time intervals. Mice receiving the liver NKs from the NLG4$^{-/-}$ animals showed a significant decrease in tumor at days 10, 12 and 14. Liver NKs from the WT animals did not alter tumor progressions. At the day of sacrifice, serum αFP levels maintained were almost not significant in all tested groups as the WT mice showed an elevations in their αFP levels and ended to similar levels of the groups probably attributed to lost effects of irradiations. Liver p53 showed to be significantly high (1.5 fold increase) in the mice groups with HCC alone while almost decreased in mice receiving liver NKs from the NLG4$^{-/-}$ to levels similar to the animals with no HCC. These results were associated with decrease in AKT only in animals receiving liver NKs from the NLG4$^{-/-}$ ($p = 0.001$).
Conclusion: p53 is a more sensitive marker for HCC tumorigenesis than αFP in C57BL/6 mice in advanced stages of tumor. The mechanism of p53-mediated repression of αFP levels may be active during hepatic differentiation and lost in the process of tumorigenesis. NK from NLG4$^{-/-}$ mice showed to decrease tumor through p53 inhibitions and decrease in AKT indicating its associations with pathways decreasing proliferations of HCC and reinforces the importance of NLG4 modulation as a therapeutic target for HCC.

P01-03YI Pivotal role of xist in regulating immune checkpoint pd-1/pd-l1 through a shared pathway between mir-194-5p and mir-155-5p in hepatocellular carcinoma

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Background and aims: Anti-programmed death therapy has thrust the immunotherapy into spotlight. Nevertheless, programmed-cell-death-ligand-1 (PD-L1) targeted therapies are yet understudied in hepatocellular carcinoma (HCC). MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) have emerged as eminent players in human pathophysiological processes. However, interaction between miRNAs and LncRNAs in immunotherapy is inadequately studied. This study aimed at exploring potential upstream regulatory ncRNAs for the immune checkpoint PD-1/PD-L1 in HCC.
Method: Bioinformatics software were used to predict PD-L1 upstream regulatory noncoding-RNAs. Twenty HCC, cirrhotic and healthy liver tissues were surgically resected from liver transplant patients. Ectopic miR-155 and miR-194 expression manipulation as well as knocking down of each of the lncRNAs XIST, TSIX (negative regulator of XIST) and MALAT-1 were performed in Huh-7-cells followed by extraction of total RNA using Trizol, then RT-qPCR was performed. RNU6B and Beta-2-microglobulin were house-keeping genes.
Results: MiR-155-5p and miR-194-5p were predicted to target PD-L1, MALAT-1 and XIST. MALAT-1 and XIST were predicted to target PD-L1 mRNA. PD-L1 and XIST were significantly upregulated in HCC biopsies compared to healthy controls, however MALAT-1 was barely detected. Ectopic miR-194 expression has markedly induced the overexpression of PD-L1, XIST and MALAT-1 ($p = 0.0219$, $p = 0.0026$, $p = 0.0135$, respectively). However, overexpression of miR-155-5p has significantly induced the upregulation of PD-L1 ($p = 0.0209$) and XIST ($p = 0.0477$), while it had a negative impact on MALAT-1 expression ($p = 0.0053$). Knocking down of XIST did show no impact on PD-L1 expression, however upon knocking down of TSIX, PD-L1 expression was elevated ($p = 0.0358$), on contrary to abolishing MALAT-1 activity that induced down regulation of PD-L1 ($p = 0.0010$). Such findings might explain the interesting role of miR-194 in elevating oncogenic PD-L1, being mediated through upregulated XIST and MALAT-1. Upon co-transfection of miR-194-5p with siMALAT-1, PD-L1 expression was elevated ($p = 0.0074$). Cotransfection of miR-194-5p with siXIST did show no impact on PD-L1 expression. Nevertheless, upon co-transfection of miR-194 with siTSIX, PD-L1 expression was upregulated ($p = 0.0067$).Interestingly, the same pattern of PD-L1 expression was revealed upon miR-155-5p co-transfection with each of the siRNAs of MALAT-1 ($p = 0.0060$), XIST and TSIX ($p = 0.0188$).
Conclusion: A novel shared upstream regulatory signalling pathway for PD-1/PD-L1 immune checkpoint between paradoxically acting tumour suppressor miR-194-5p and onco-miR-155-5p is on the horizon, through XIST expression modulation. Such key regulators could be employed as therapeutic targets for HCC as well as having an implication in clinical diagnosis.

P01-04YI endocan versus alphafetoprotein as a novel biomarker in hepatitis C related cirrhosis with hepatocellular carcinoma

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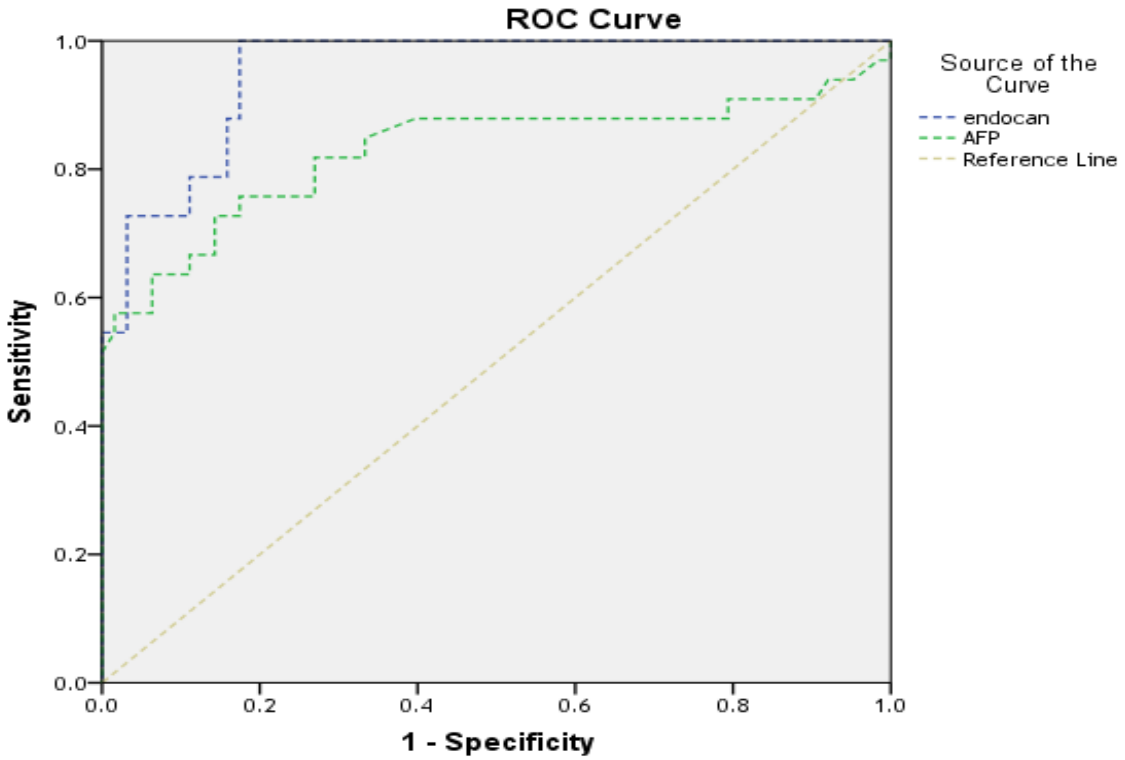
Background and aims: In Egypt, the prevalence of hepatitis C virus infection is high (about 20 %). HCC is the major cause of death in patients with chronic hepatitis C virus infection. HCC is the most common primary liver cancer and the third common cause of cancer related death (14.8 %). Alpha Fetoprotein (AFP) is the most commonly used tumor biomarker for the early detection and clinical follow-up of patients but its low positive rate, false-positive and false-negative results limits its value in diagnosis of HCC. Endocan is a 50 kilo Dalton soluble proteoglycan that is produced and secreted by tumor vascular endothelial cells. Recent studies showed that endocan is overexpressed in HCC tissues and sera and has been associated with tumor progression and poor outcomes. We aim to validate endocan level in HCV cirrhotic patients with or without HCC compared to AFP.

Method: Sixty six Egyptian patients with chronic hepatitis C (CHC) were divided into 2 groups and thirty healthy subjects as a control group, measurement of serum endocan and AFP level were done.

Results: Serum endocan level was significantly high in HCC patients with cutoff point ≥3.59 ng/ml with 100 % sensitivity, 83 % specificity which is superior to AFP level in which cutoff point was ≥14.3 ng/ml with 82 % sensitivity and 73 % specificity.

Conclusion: Serum endocan level can be considered a good diagnostic marker in HCC on top of CHC infection as compared to AFP

Figure:



P01-05YI The effect of socio-economic deprivation and distance to travel in the survival of patients with Hepatocellular carcinoma in Liverpool region (UK)

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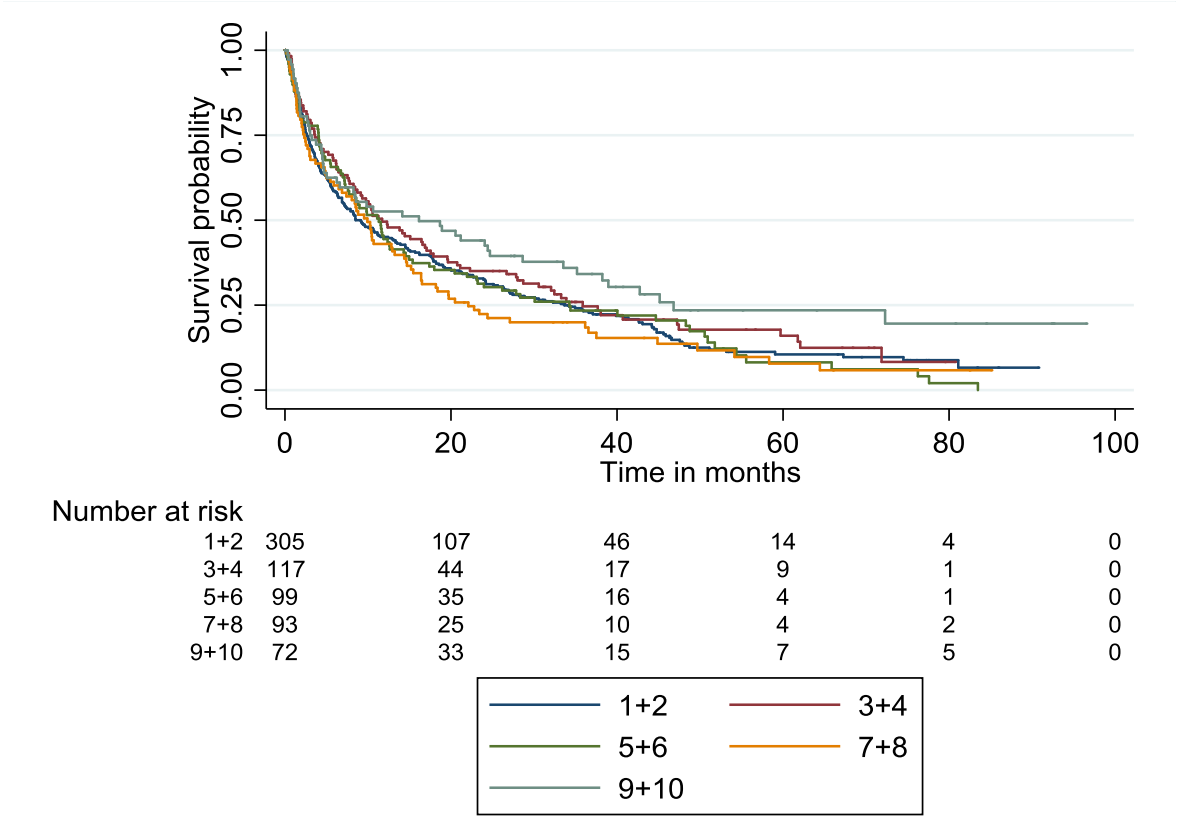
Background and aims: HCC is known to be the 6th most common and 2nd most common cause of cancer related death and the incidence and mortality continue to increase in UK and worldwide. The aim was to investigate if socio economic deprivation status played a role in survival of patients with HCC and if distance and time to travel to treatment centre had any effect.
Method: A 6-year retrospective case series analysing all patients diagnosed with HCC in North west of England (Liverpool region) from January 2010-December 2015 was conducted. Patients were diagnosed on international recognised criteria using BCLC staging. Index of multiple deprivation (IMD) which is the official measure of relative deprivation of small areas in England was calculated on each patient based on the postcode they resided at the time of diagnosis. Distance in kms and time to travel to treatment centres was collected from patient's residential post code. Kaplan-Meier curves, log-rank test statistics and median overall survival estimates along with 95% CI were reported. Univariate and multivariable Cox proportional hazards models were generated for covariates of interest. Hazards proportionality assumption was assessed.
Results: 687 patients were diagnosed with HCC, Male = 529, Female = 158, median age in years was 69 (IQR 61-77). BCLC stage at time of diagnosis 0+A = 212, B = 162, C = 169 and D = 144 patients respectively. The median survival of patients in the least deprived group = 16.1 months (95% CI-6.2-28.6) compared to the most deprived group = 9.1 months (95% CI-6.9-12.9). On univariate analysis, patients in the least deprived background were 28% more likely to survive from HCC when compared to patients from the most deprived areas, (HR 0.72[95% CI-0.53-0.97]), p = 0.032]. IMD did not make it to the multivariable model when adjusting for other covariates of interest. The distance and time to travel to treatment centre was not statistically significant on univariate analysis.
Conclusion: Our study showed that deprivation status played a role in the survival of patients with HCC in a univariate setting. This could be due to the lack of awareness of liver cancer in the most deprived groups, more risky behavior among individuals in these areas and lack of a regular surveillance program as recommended by EASL and NICE guidelines.

Figure:


Survival probability
0.00
0.25
0.50
0.75
1.00
0
20
40
60
80
100
Time in months
Number at risk
1+2 305 107 46 14 4 0
3+4 117 44 17 9 1 0
5+6 99 35 16 4 1 0
7+8 93 25 10 4 2 0
9+10 72 33 15 7 5 0
1+2
3+4
5+6
7+8
9+10

P01-06 Anticlonogenic and apoptotic effect of metformin on cholangiocarcinoma cell line

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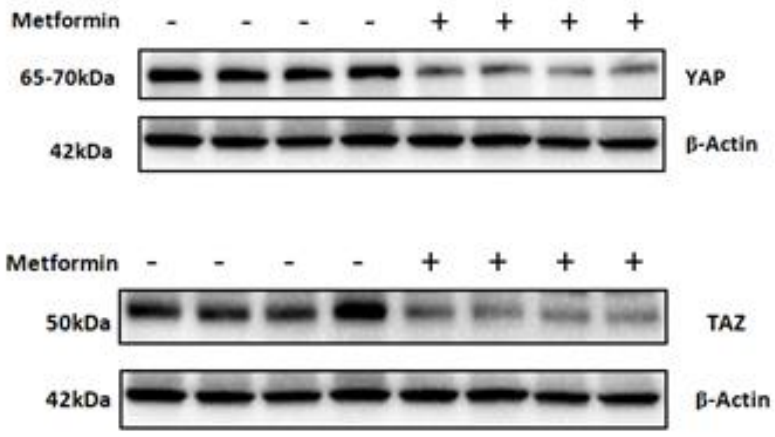
Background and aims: Cholangiocarcinoma (CCA) is the second primary liver cancer type after hepatocellular carcinoma. Metformin is an oral antidiabetic drug. Emerging data have revealed that metformin might have protecting effect against liver cancer development. Metformin might express anticarcinogenic effect by different mechanisms. The Hippo signaling pathway has recently emerged as a pivotal player in cancer biology. In this study, the molecular mechanisms of metformin's effect on carcinogenesis on extrahepatic CCA cell line (TFK cell line) were assessed by considering colony formation assay, apoptosis and Yes-associated protein (YAP)/transcriptional coactivator with a PDZ-binding domain (TAZ) proteins in vitro.
Method: TFK cell line was cultured and maintained in RPMI-1640 medium and supplemented with 10 % (*v/v*) fetal bovine serum and 1 % penicillin G-streptomycin. The cells were cultured in a humidified atmosphere of 5 % CO_2 at 37 ° C. Cell line was treated with 4.5 mmol/L metformin for 48 hours. YAP/TAZ protein levels were measured by western blotting. Colony forming unit was used for colony count. Apoptosis was revealed by Annexin V Dead Cell Kit.
Results: We found that metformin significantly inhibited the cellular proliferation in a time-dependent way. Metformin statistically significantly decreased both levels of YAP/TAZ ($p < 0.001$) proteins and colony formation ($p < 0.014$). Early, late and total apoptosis statistically increased after metformin treatment, respectively ($p < 0.006$, $p < 0.012$, $p < 0.003$).
Conclusion: Present study revealed that metformin might have protective effect on cholangiocarcinoma by inhibiting hippo pathway as shown by decreased levels of YAP/TAZ proteins and inducing apoptosis in TFK-1 cells. Activation of the oncogene YAP has been shown to be related to many cancer progression and associates with poor prognosis and metastasis. Therapeutically, metformin might be useful in the treatment of CCA.
Figure: YAP/TAZ proteins in TFK cell line.



P01-07YI Sorafenib-induced pancreatic atrophy and its clinical implication in patients with hepatocellular carcinoma

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Background and aims: Sorafenib is the frontline therapy for advanced hepatocellular carcinoma (HCC). Pancreatic toxicity, from asymptomatic elevation of pancreatic enzymes to overt acute pancreatitis, is a common adverse even. Pancreatic atrophy has been reported as a relatively infrequent event. However, the actual prevalence of sorafenib-induced pancreatic atrophy, its clinical impact and its possible prognostic role remain elusive. Our aim to prospectively asses the pancreatic volume at the baseline and six months after the start of sorafenib. We also looked for correlations between pancreatic volume reduction, prevalence of sorafenib-related diarrhoea, and overall survival (OS).
Method: We evaluated 52 consecutive patients who started sorafenib for hepatocellurar carcinoma in our outpatient clinic. As part of the routine clinical examinations, these patients underwent a thorax-abdomen-pelvis computed tomography: a) within 2 weeks before the start of sorafenib; b) every two months after the first dose (± 2 weeks).Pancreatic volume and response to sorafenib were evaluated at each examination. Clinical evaluations were performed a week after each CT. Clinicians were blinded to the results of pancreatic volume assessment.
Results: Pancreatic volume was significantly lower 6 months after the start of sorafenib compared to the baseline ($p < 0.01$). In detail, 29 patients (55.8%) had a volume loss >10%. The prevalence of diarrhoea was similar in patients with and without volume loss >10% (48.2 vs 30.7%, $p = 0.403$). A significant volume loss was not associated with an higher rate of lipase elvation (34.5 vs 34.6%, $p = 1.000$). Either, there were no correlation with the median average daily dose of sorafenib or with the OS (median 9.6 vs 9.0 months, $p = 0.435$).
Conclusion: Reduction of pancreatic volume is a relatively common event in sorafenib-treated patiens, however it has no significant clinical implications.

P01-09 Treatment of BCLC-B hepatocellular carcinoma with transarterial chemoembolization with novel polyethylene glicol doxorubicin drug-eluting microspheres: a pharmacokinetic study

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Background and aims: Transarterial chemoembolization (TACE) is the established treatment of BCLC B hepatocellular carcinoma (HCC), using conventional TACE as well drug eluting microspheres (DEM). DEM optimize the TACE procedures by loading drugs and delivering them directly into the HCC in a controlled fashion, achieving high intratumoral concentrations while maintaining a low plasma concentration of drugs.

This study assessed the pharmacokinetic (PK) profile, safety and efficacy of novel polyethylene glycol highly compressive DEM (LifePearl®) in the treatment of HCC when loaded with different doses of doxorubicin.

Method: Multicenter, prospective study based on 2 cohorts. Cohort I was a dose escalation study with 3 groups of 3 patients receiving respectively 75mg, 100mg and 150mg of doxorubicin. After verifying that the highest dose was safe, cohort II (still under follow-up) consisted in 16 additional patients treated at 150mg.

Blood samples were collected at pre-specified time points (figure 1) and analyzed by independent laboratory for all enrolled patients. Maximum systemic concentration of doxorubicin (Cmax), and area under the curve (AUC) were calculated for each of the dose cohorts (≤75mg, 76-100mg and 101-150mg doxorubicin)

Safety was evaluated within 30 days after procedure. Response was evaluated according to EASL and mRECIST criteria.

Results: Twenty-five patients were enrolled (68% males), mean age was 73.8 ± 8.5 years. 92 % and 8% were Child Pugh A and B respectively. Mean number of target lesions was 1.8 ± 1.3 and mean diameter was 72.6 ± 45.3mm. Cmax was 286.7 ± 220.1, 157.1 ± 94.6 and 227.8 ± 139.5 (ng/ml) and AUC was 1, 040.4 ± 671.4, 593.9 ± 161.4 and 1, 938.5 ± 875.6 (ng x hours)/ml for patients receiving ≤75 mg, 76-100mg and 101-150mg of doxorubicin, respectively.

Figure 1 represents the Cmax for each dose cohort at different time points. No statistical differences between the groups were observed.

Eighteen adverse events of grade 3 or more were reported in 12 patients. Only 2 adverse events were considered as possibly related to LifePearl® by the investigator.

Objective response (70%) and disease control (85, 5%) (no difference between EASL and mRECIST) were achieved in 20 patients with at least one imaging control available.

Conclusion: TACE using LifePearl® loaded with doxorubicin is safe and effective for the treatment of HCC with very low systemic concentration of doxorubicin detected in plasma 6h after the procedure.

Figure:

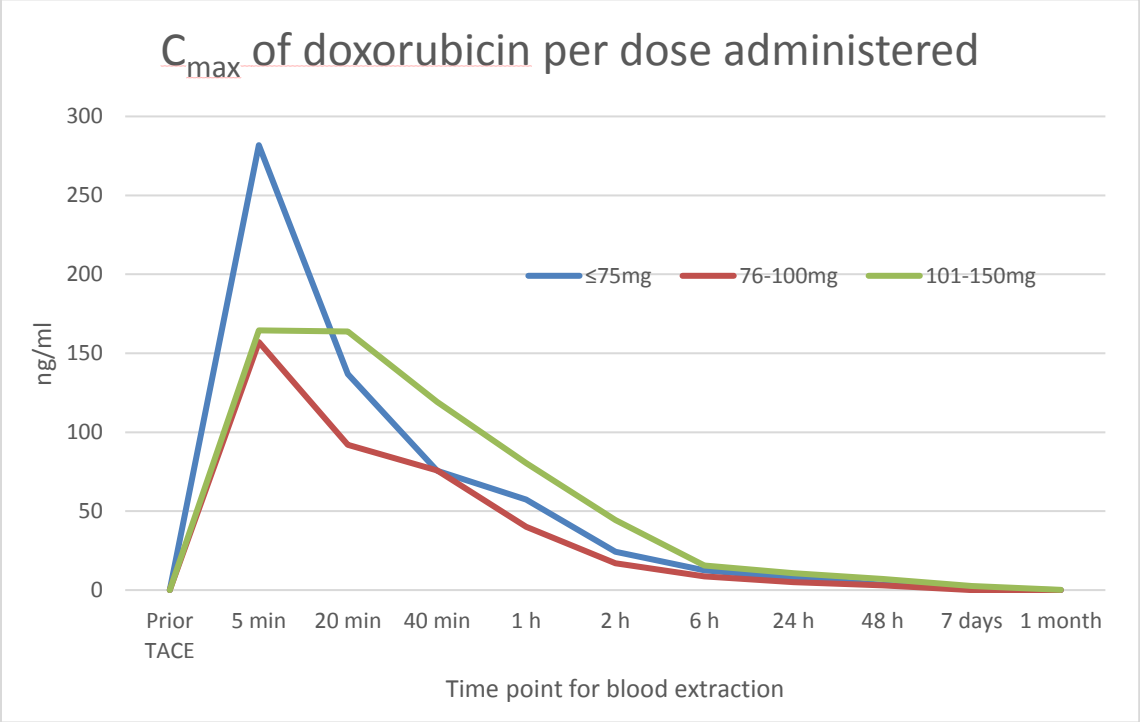

Figure 1: C_{max} of doxorubicin at different time points of blood extraction for each dose cohort (≤75 mg, 76-100mg and 101-150mg) of doxorubicin

P01-10YI beta-catenin signaling controls NKG2D ligands expression in liver tumorigenesis

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Background and aims: Hepatocellular carcinoma (HCC) has emerged as the second leading cause of cancer-related death worldwide. Due to the advanced stages of HCC at the time of diagnosis, conventional treatments for solid tumors frequently end with treatment failure, recurrence, or poor survival. However, due to the impressive role of the immune microenvironment during tumorigenesis, approaches targeting immune effectors represents an exciting prospect for therapeutic.
In these lines, our goal is to understand immunosurveillance mechanisms taking place during HCC using both murine models and human samples.
These mechanisms are based on the expression of ligands for example after oncogenic stress on epithelial cells that are recognized by the receptor on immune cells. We focused to study NKG2D system described as very efficient anti-tumoral process in several tumors.
Method and Results: First, we monitored the expression of NKG2D ligands in a vast cohort of HCC patients ranged according to HCC classification groups. Genomic functional studies helped to define two major HCC tumor groups based on chromosomal stability: G1/G2/G3 groups associated with high chromosomal instability, inflammation and poor prognosis (*TP53* mutated) ; G4/G5/G6 groups associated with low chromosomal instability and good prognosis (G5/G6 *CTNNB1* mutated)
We showed that NKG2D ligands mRNA expression was higher in HCC tumors harboring chromosomal instability (G1/G2/G3) compared to G4/G5/G6 groups suggesting that NKG2D ligands expression associates with chromosomal instability.
Accordingly, we validated these results in our murine HCC models, one mimicking G1/G2/G3 groups of aggressive tumors (DEN induced HCC model) and the other harboring *ctnnb1* mutated HCC tumors mimicking G5/G6 groups.
Moreover, we observed a strong downregulation of NKG2D ligands both at the mRNA and the protein level in our *ctnnb1* mutated HCC.
In addition, we observed the same behavior in other β-catenin activated context e.g. in the Apc KO mouse model that recapitulates a drastic activation of β-catenin signaling and β-catenin mutated αML cell line by CRISPR-Cas 9 technology. These results evidenced that β-catenin signaling controls negatively NKG2D ligands expression.
To get into the mechanism, we identified by ChIP-seq immunoprecipitation that β-catenin directly controls NKG2D ligands.
Conclusion: Collectively, these results identified β-catenin as a strong regulator of NKG2D ligands expression in the liver.
We are currently investigating whether the reintroduction of NKG2D ligands expression in *ctnnb1* dependent liver tumorigenesis will worsen the course of tumorigenesis in terms of inflammation, proliferation and grade of tumors.

P01-11 ESM-1 as a marker of macrotrabecular-massive hepatocellular carcinoma

Julien Calderaro[1 2], Lea Meunier[3], Marouane Boubaya[4], Cong Trung Nguyen[1], Alain Luciani[5], Helene Regnault[6], Giuliana Amaddeo[6], Jean Charles Nault[7], Frédéric Oberti[8], Sophie Michalak[9], Mohamed Bouattour[10], Valérie Paradis[11], Georges-Philippe Pageaux[12], Jeanne Ramos[13], Nathalie Barget[14], Boris Guiu[15], Valerie Vilgrain[16], Christophe Aubé[17], Nathalie Ganne-Carrié[7], Jean-Michel Pawlotsky[2], Olivier Seror[18], Jessica Zucman-Rossi[3], Marianne Ziol[14]

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Background and aims: Macrotrabecular-massive hepatocellular carcinoma (MTM-HCC) is a novel morphological subtype of HCC associated with early relapse after resection or percutaneous ablation, independently of classical clinical, biological and radiological prognostic factors. The aim of the present study was to identify immunohistochemical markers of MTM-HCC, to ease its diagnosis and implementation into clinical practice.

Method: To identify potential biomarkers of MTM-HCC, we first analyzed gene expression profiling data from the TCGA study and further selected two candidate biomarkers. Performance of both biomarkers for diagnosis of MTM-HCC was further tested by immunohistochemistry in a discovery and a validation series of 67 and 117 biopsy samples.

Results: Analysis of RNA sequencing data showed that MTM-HCC was characterized by a high expression of neoangiogenesis related genes. Two candidate biomarkers, Carbonic Anhydrase IX (CAIX) and Endothelial Specific Molecule 1 (ESM-1), were selected. In the discovery series, sensitivity and specificity of ESM-1 expression, by stromal endothelial cells for the detection of MTM-HCC was 96% (24/25), and 83% (35/42), respectively. Specificity and sensitivity of CAIX was 88% (37/42) and 52% (13/25), respectively. In the validation study, sensitivity and specificity of ESM-1 for the identification of MTM-HCC were 92% (11/12) and 87% (89/102), respectively. Interobserver agreement for ESM-1 assessment was good (Cohen Kappa = 0.77).

Conclusion: Using a molecular driven selection of biomarkers, we identified ESM-1 as a micro environment immunohistochemical marker of MTM-HCC that may help the implementation of a morpho-molecular subtyping into clinical staging systems.

P01-12YI Analysis and characterization of mitochondrial DNA mutations in The Cancer Genome Atlas hepatocellular carcinoma cohort

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Background and aims: Hepatocellular carcinoma (HCC) is the most common primary hepatic malignancy and represents the second cause of cancer related death worldwide, characterized by high recurrence rates and poor survival, even when detected and treated at its early stages. Mitochondrial mutations have been known to play a role in carcinogenesis, but to date, few studies correctly prioritize the variants discovered. Thus, we aimed to identify and analyze mtDNA mutations in the HCC dataset from The Cancer Genome Atlas (TCGA) consortium.
Method: Whole exome sequencing fastq files from 376 TCGA-HCC patients (paired tumor, non-tumor tissues) were processed to reconstruct the mtDNA genomes using the MToolBox automated pipeline. Pairwise comparison between blood/normal solid tissue and tumor was performed in order to identify the potentially germline and tumor-specific somatic mtDNA variants. Information regarding the variability and pathogenicity of the variants were obtained from HmtVar database.
Results: The assembly of the mitochondrial reads showed an adequate coverage and quality for 104 patients. Variants were classified as pathogenic based on the allele frequency and disease score. After discarding the germline variants used in haplogroup classification and applying a heteroplasmic fraction (HF) >0.4, 374 variants remained, among which 132 (35%) were synonymous, 60 (16%) non-synonymous and 189 (50%) were variants belonging to the non-protein coding class. Among these, 14 were pathogenic/likely-pathogenic and out of the nine non-protein coding mutations mapped in tRNA genes only one was classified as pathogenic. A total of 320 somatic mtDNA variants were found. Seventy-nine variants had HF>0.4 and were distributed in 46 patients (44.2%). Out of these, 8 (10%) were synonymous substitutions, 22 (28%) non-synonymous and 50 (63%) non-protein coding. Among all the non-synonymous variants found, 15 resulted pathogenic. Among the non-protein coding class, 5 out of 42 variants (11.9%) mapped in genes encoding for tRNAs, and only one was classified as pathogenic. The burden of mtDNA variants did not corelate with the survival of these patients.
Conclusion: Although a high number of mtDNA variants was found in the TCGA-HCC cohort, few remained after applying the pathogenicity criteria. mtDNA mutations could cause mitochondrial dysfunction and contribute to hepatocarcinogenesis but do not predict overall survival.

P01-13YI Time varying mHAP III is the most accurate score in predicting survival in patients with hepatocellular carcinoma (HCC) undergoing transarterial chemo-embolization (TACE)

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Background and aims: TACE is the most widely used treatment for patients with unresectable HCC. The prognosis of these patients is extremely variable due to differences in liver function, tumor burden and performance status and a median survival of around 40 months has been reported for well-selected candidates. Many prognostic scores have been proposed to identify those more likely to benefit from TACE. Moreover, albumin-bilirubin (ALBI) and platelet-albumin-bilirubin (pALBI) grades have not been specifically evaluated in patients undergoing TACE.
An important confounding effect is that TACE is often repeated several times, but there are no studies evaluating prognostic scores as time-varying variables, i.e. recalculating the scores before each TACE procedure. The aim of this study was to compare different prognostic and staging systems in patients undergoing TACE in a large series of patients with HCC.
Method: We retrospectively evaluated the accuracy of HAP, mHAP II, mHAP III, ALBI, and pALBI in estimating overall survival (OS) in patients with HCC undergoing TACE. We considered 1610 TACE performed in 1058 patients recorded in the Ita.Li.Ca database from 2008 trough 2016. Exclusion criteria were CPT score>7 and/or refractory ascites. We carried out a time-dependent analysis to consider prognostic scores as time-varying variables, and calculated OS from the time of TACE to the time of the subsequent treatment. The total follow-up time for each patient was therefore split into several observations accounting for each TACE procedure, where appropriate. Values of the likelihood ratio test (LRT) and Akaike information criterion (AIC) were used to compare different systems.
Results: The median survival of the 1058 enrolled patients undergoing 1610 TACE procedures was 36 months. Comparing LRT and AIC values of the prognostic scores, mHAP III achieved the highest $\chi 2$ and lowest AIC values (39, 6 and 4638 respectively, $p < 0.0001$), indicating an improved predictive performance compared with HAP ($\chi 2$ 16.3, AIC 4665, $p = 0.0010$), mHAP ($\chi 2$ 19.9 AIC 4662, $p = 0.0003$), ALBI ($\chi 2$ 4.34, AIC 4673, $p = 0.0373$), whereas pALBI was not significantly different ($\chi 2$ 1.47, AIC 4676, $p = 0.2254$). The prognostic performance of mHAPIII was also superior to that of other HCC prognostic systems (BCLC, ITALICA, CLIP, MESH, MESIAH, JIS and HKLC). In time-varying multivariable Cox proportional hazards model, mHAP III maintained an independent effect on OS (hazard ratio 1.34, 95% CI 1.15-1.56). Other significant variables in the multivariable model resulted age, alcoholic etiology, radiologic response to TACE, and performing ablation or surgery after TACE.
Conclusion: In a large series of patients with HCC undergoing TACE, mHAP III was identified as the most accurate scoring system for predicting OS, using an innovative, time-dependent analysis of patients comprised in the Ita.Li.Ca. database.

P01-14 Oncostatin M induces increased invasiveness and angiogenesis in hepatic cancer cells through HIF1-alpha-related release of VEGF-A

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Background and aims: Oncostatin M (OSM), a pleiotropic cytokine belonging to the interleukin-6 (IL-6) family, can modulate hypoxia-dependent liver processes (development, regeneration and angiogenesis), contributing to chronic liver disease progression and hepatocellular carcinoma (HCC) development. Recently, hypoxia, as an independent signal operating through hypoxia-inducible factors (HIFs), has been shown to induce epithelial-to-mesenchymal transition (EMT) in cancer cells, including HepG2 cells. In this connection, OSM-related signaling pathway has been reported to up-regulate HIF1α and switch on EMT program. In this study we investigated in vivo and in vitro, the relationships between OSM, expression of vascular endothelial growth factor A (VEGF-A), and increased invasiveness.
Method: EMT, invasiveness, angiogenesis and signal transduction pathways were analysed by integrating morphological, molecular and cell biology techniques in the following experimental models: a) cohort of HCC patients b) HepG2 cells exposed to human recombinant OSM (hrOSM) or stably transfected in order to overexpress human OSM (H/OSM) or empty vector; c) murine xenograft.
Results: 1) Oncostatin M can induce EMT in both in vitro models (HepG2 cells exposed to human recombinant OSM or H/OSM) and stimulate invasiveness through VEGF release in culture medium and VEGF-dependent activation of PI-3K, ERK1/2, and p38MAPK signalling pathways; the use of specific pharmacological inhibitors against PI-3K, ERK1/2, p38MAPK signaling pathways as well as the use of neutralizing antibody raised against Flk-1 (VEGF receptor type 2) or of a specific inhibitor of Flk-1 results in decrease of invasiveness induced by conditioned medium collected by HepG2 cells treated with hrOSM for 48hrs; 2) xenograft experiment shows an anti-proliferative effects of Oncostatin M, confirmed also in vitro (BrdU assay and cell cycle analysis); 3) oncostatin M seems to promote angiogenesis through OSM-dependent production of VEGF; 4) the highest levels of OSM transcripts correlate in HCC specimens with the highest rate of early tumor recurrence.
Conclusion: OSM, expressed in human HCC, can induce EMT and increased invasiveness in human hepatic cancer cells through a mechanism involving HIF1α-dependent release of VEGF-A.

P01-15YI Milticentric prospective study of validation of angiogenesis polymorphisms in hepatocellular patients treated with sorafenib. Interim analysis of INNOVATE study

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Background and aims: Although sorafenib is the upfront standard care of treatment for advanced hepatocellular carcinoma (HCC), molecular predictors of efficacy have not been identified yet. In the ePHAS study we analyzed three *eNOS* polymorphisms and at univariate analysis, patients with *eNOS*-786 (rs2070744) TT genotype had significantly shorter median Progression Free Survival (PFS) and Overall Survival (OS) compared to those with other genotypes. In the ALICE-1 and ALICE-2 studies, *VEGF-A*, *VEGF-C, VEGFR* and *HIF-1α* polymorphisms resulted independent prognostic factors for PFS and OS at multivariate analyses. On the basis of these preliminary results, our aim is to validate in a prospective study these data in patients with HCC treated with sorafenib (NCT02786342).

Method: This is a prospective Italian multicenter study, that includes 160 HCC patients receiving sorafenib. For this interim analysis we analyzed *eNOS-786* polymorphism on 119 patients. *eNOS*-786T>C was analyzed by Real Time PCR in relation to the primary end point (OS). Event-time distributions were estimated using the Kaplan-Meier method and survival curves were compared using the log-rank test.

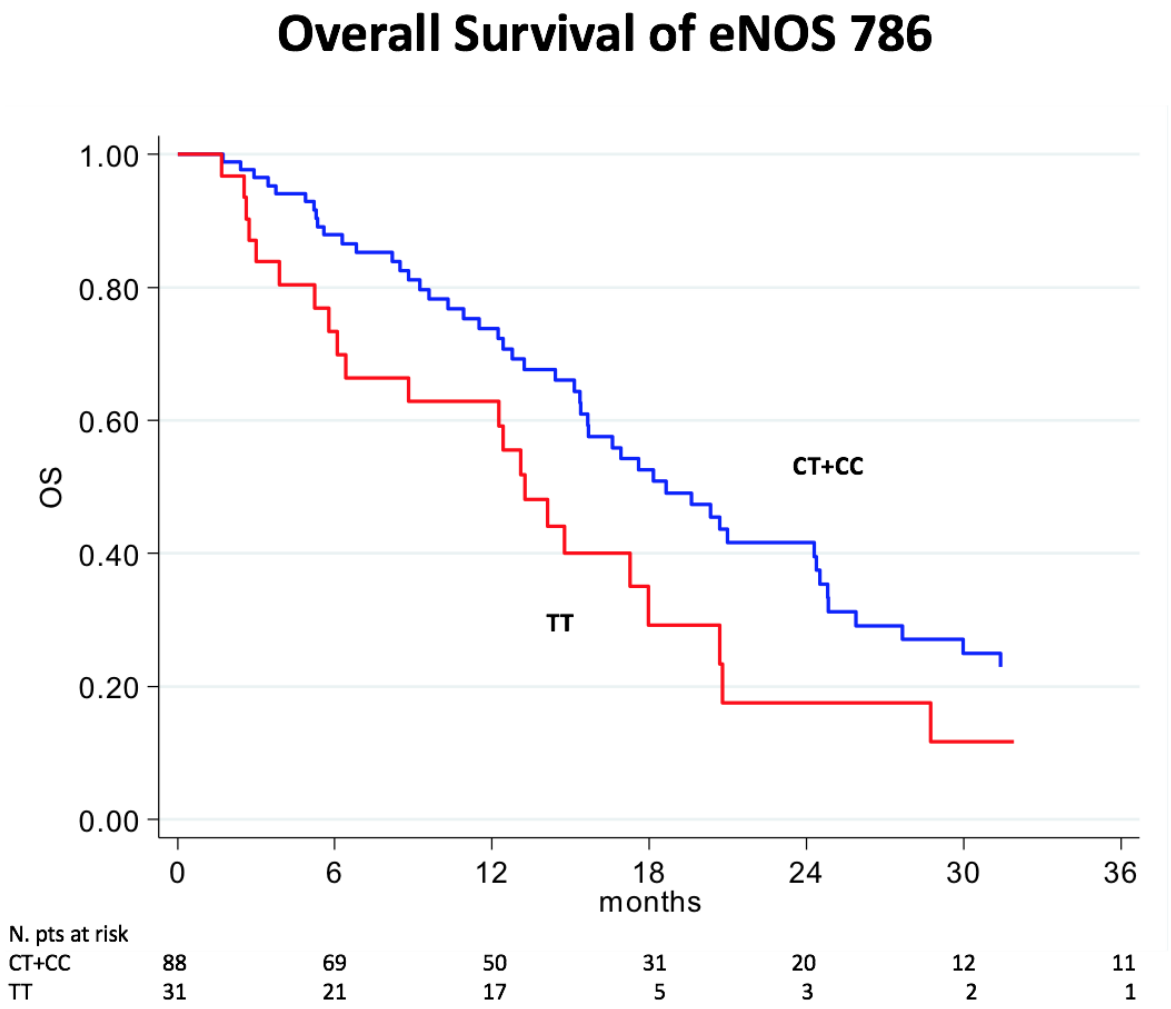
Results: 119 HCC patients (102 males and 17 females), prospectively treated with sorafenib from May 2015 to September 2018 were included. Median age was 69 years (range 28-88 years). 95 patients had Child-Pugh A and 23 had Child-Pugh B7. 42 had BCLC-B and 77 patients had BCLC-C.

At univariate analysis, we confirmed that *eNOS*-786 TT genotype were significantly associated with a lower median OS than the other genotypes (13.3 vs 18.7 months respectively $p = 0.021$, HR 1.96, 95% CI 1.08-2.94 $p = 0.023$). Moreover, patients carrying a TT genotype for *eNOS*-786 showed a lover percentage of Disease Control

Conclusion: This preliminary date confirms the prognostic role of *eNOS*-786 in advanced HCC patients treated with sorafenib. Previous studies have suggested that DNA variant at the *eNOS* gene can quantitatively regulate eNOS expression. The point variation at nucleotide -786bp was associated with a significant reduction in the *eNOS* gene promoter activity resulting in lower levels of eNOS mRNA, eNOS protein and enzyme activity.

In conclusion, our data show that *eNOS* polymorphisms may identify patients who are more likely to benefit from sorafenib treatment.

Figure:


Overall Survival of eNOS 786
1.00
0.80
0.60
0.40
0.20
0.00
OS
CT+CC
TT
0
6
12
18
24
30
36
months
N. pts at risk
CT+CC 88 69 50 31 20 12 11
TT 31 21 17 5 3 2 1

P01-16YI Cancer stem cell sub-population drives resistance to anti-angiogenic therapies in hepatocellular carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) is highly vascular tumor where activation of neo-angiogenic processes during disease progression is frequently associated with poor clinical outcome. Consequently, inhibition of neo-angiogenesis is an effective treatment strategy for advanced HCC. However, development of chemoresistance is observed in the majority of 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 different anti-angiogenic therapies in HCC and define concomitant adaptive molecular changes.
Method: Four HCC cell lines and two primary HCC isolates were exposed to sorafenib and sunitinib 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.
Results: Both treatments effectively reduced oncogenic properties in all investigated HCC cells. However, sustained anti-proliferative effect after treatments was observed in only one cell line. In three other lines initial treatment effect was subsequently followed by rapid re-growth thereby mimicking the responses observed in patients. Interestingly, two cell lines showed differential response to applied drugs, showing anti-proliferative effects to sorafenib, while relapse formation occurred after sunitinib treatments. 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 both drugs 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 as well as multidrug resistance ABC transporters.
Conclusion: Our model recapitulates features of drug resistance observed in human HCC patients. Resistance to anti-angiogenic therapies 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.

P01-17YI Genetic variation in death receptor domain 4 (DR4) gene and the susceptibility to hepatitis-c related hepatocellular carcinoma

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Background and aims: Hepatocellular Carcinoma (HCC) is a leading cause of cancer mortality worldwide particularly in Egypt. The role of apoptosis in tumorigenesis has been well-documented and resistance to apoptosis is a hallmark of cancer. The association between death receptor 4 (DR4) genetic variants and HCC risk is still under investigations. The aim of this work is to study the possible link between DR4 gene polymorphism and the susceptibility to HCC
Method: The current case control study was conducted on 200 participants; 100 HCC patients on top of HCV-related liver cirrhosis and 100 HCV-related cirrhosis patients without HCC. Patients were recruited from Multidisciplinary HCC clinic, Cairo University and Theodor Bilharz Research Institute from January 2016 to June 2017. Additionally, 150 age and gender matched healthy controls were included . Genotyping of DR4-C626G, -A683C and DR4-A1322G single nucleotide polymorphisms (SNP) by TaqMan allelic discrimination assay on Real Time PCR system (Applied Biosystems, Foster City, USA) containing probes for both alleles.
Results: DR4-A1322G polymorphic genotypes (AG and GG) were significantly higher in HCC and cirrhotic patients than controls. The AG genotype conferred two folds increased risk of HCC (OR = 2.34, 95%CI = 1.56-3.51). Risk increased to three folds for the GG genotype (OR = 3.51, 95%CI = 2.33-5.28). The frequency of DR4 -C626G and -A683C SNPs in HCC and cirrhotic patients were not significantly different from the controls. Combined genotype analysis showed that co-inheritance of the polymorphic variants of DR4 -C626G and -A1322G conferred 9 folds increased risk of HCC (OR = 9.34, 95%CI = 3.76-23.12), and the risk increased to be 12 folds when DR4 -A683C and -A1322G variants were co-inherited (OR = 11.9, 95%CI = 4.82-29.39). Co-existence of the variant genotypes of the three SNPs conferred almost 10 fold increased risk of HCC (OR = 9.75, 95%CI = 1.86-51.19)
Conclusion: The G allele of DR4 -A1322G could be considered as a novel independent molecular predictor for HCV-related HCC.

Figure:

SNP	Genotypes	Controls (n = 150)	HCV patients (n = 100)	HCC patients (n = 100)	P value		
					Controls vs HCV	Controls vs HCC	HCV vs HCC
DR4-A1322G	**AA**	106 (70.7%)	45 (45%)	17 (17%)	1 (Reference)		
	AG	38 (25.3%)	41 (41%)	37 (37%)	**0.049**	0.1	0.6
	GG	6 (4%)	14 (14%)	46 (46%)	**0.02**	**0.001**	**0.001**
	Allele A	0.83	0.65	0.35	1 (Reference)		
	Allele G	0.17	0.35	0.65	**0.001**	**0.001**	**0.001**

SNP		Control group (n = 150)	HCC patients (n = 100)	OR	95% CI	P value
A1322G (rs2230229) DR4	**AA**	106 (70.7%)	17 (17%)			
	AG	38 (25.3%)	37 (37%)	**2.34**	1.56-3.51	**0.049**
	GG	6 (4%)	46 (46%)	**3.51**	2.33-5.28	**0.02**
	AG + GG	44 (29%)	83 (83%)	**11.76**	6.27-22.07	**0.001**
	Allele A	0.83	0.35	1 (Reference)	**Allele A**	0.83
	Allele G	0.17	0.65	**9.09**	5.97-13.83	**0.001**

P02-01YI The role of Albumin-Bilirubin grade (ALBI) and Neutrophil-lymphocyte ratio (NLR) in predicting survival in patients with Hepatocellular carcinoma (HCC)

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Background and aims: Hepatocellular carcinoma is known to be the 6th most common and 2nd most common cause of cancer related death. The underlying liver function measured by albumin and bilirubin is an important objective measure in treating and managing patients with HCC.NLR has been used to assess the prognosis of patients with cancers. We aim to investigate the role of ALBI score and NLR on survival of patients with HCC in our cohort.
Method: A 6-year retrospective case series analysing all patients diagnosed with HCC in North-west England (Liverpool region) from January 2010-December 2015 was collected. Blood parameters were collected at the time of diagnosis. A NLR cut-off value of 3 was used based on the median overall cohort. Kaplan-Meier curves, log-rank test statistics and median overall survival estimates along with 95% CI were reported. Univariate and multivariable Cox proportional hazards models were generated for covariates of interest. Hazards proportionality assumption was assessed.
Results: 687 patients were diagnosed with HCC, Male = 529, Female = 158, median age in years was 69 (IQR 61-77). BCLC stage at time of diagnosis 0+A = 212, B = 162, C = 169 and D = 144 patients respectively. Median overall survival of patients with ALBI grade 1 = 28.3 months (95% CI-23.4-37.5) compared to ALBI grade 2 = 6.8 months (95% CI-5.2-8.4). In Univariate cox modelling, patients with ALBI grade = 2 were 2.2 times likely to die compared to ALBI grade = 1 (HR 2.27[95% CI-1.88-2.73], p <0.001). In a multivariate setting with BCLC stage, treatment, performance status and AFP, patients with ALBI grade = 2 were 1.5 times likely to die compared to ALBI grade = 1, (HR 1.47[95% CI-1.13-1.90], p = 0.004). Median overall survival of patients with NLR <3 = 24.2 months (95% CI -19.9-30.1) compared to NLR ≥3 = 8.2 months (95% CI-6.2-9.5), log-rank test p <0.001. In univariate cox modelling, patients with NLR ≥3 were 1.7 times more likely to die when compared to patients with NLR <3. (HR 1.71[CI-1.42-2.05], p <0.001). NLR did not make it to the multivariable model when adjusting for other covariates of interest.
Conclusion: The proinflammatory response of NLR and ALBI grade are simple bedside scores which can be calculated using routine blood tests and can predict survival of patients with HCC. Treatment and management of HCC patients need to be tailored according to liver function in combination with stage of disease and performance status. The NLR helps in determining the immune system changes and its role can be considered in predicting survival for future studies given the role of Immunotherapy in treating HCC patients in recent clinical studies.

P02-02YI New Multi Inflammation (MII) in advanced hepatocellular carcinoma patients receiving sorafenib

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Email: andrea.casadei@irst.emr.it

Background and aims: Several inflammation and immune-based prognostic scores, such as lymphocyte count, neutrophil-lymphocyte ratio (NLR), and systemic immune-inflammation index (SII), have been developed to predict survival and recurrence in cancers, including HCC.
We evaluated the potential role of new multi inflammation indicators (MII) as predictors of outcome in HCC patients treated with sorafenib.
Method: 170 patients with HCC (24 BCLC B and 146 BCLC C) consecutively treated with sorafenib between March 2008 and August 2018 were enrolled. Information on neutrophil, lymphocyte and platelet counts was obtained from blood tests carried out the week before the start of treatment. The MII-1 was calculated as the ratio between the absolute neutrophil count and the lymphocyte count (NLR) x high sensitivity protein C reactive (hs-PCR); MII-2 was calculated as the ratio between the absolute platelet count and the lymphocyte count (PLR) x hs-PCR; MII-3 was calculated as platelet count × NLR (SII) x hs-PCR.
PFS and OS were estimated by the Kaplan-Meier method and curves were compared by the log-rank test. Unadjusted and adjusted hazard ratios (HRs) by baseline characteristics (age, gender, etiology, ECOG performance status) were calculated using the Cox proportional hazards model.
We also conducted landmark analyses to reduce possible confounding by time on treatment by assessing the impact of change in SII; NLR and PLR at 1 month landmark time on survival outcomes. X-tile 3.6.1 software (Yale University, New Haven, CT) was used to determine the cutoff value for baseline levels of each II.
Results: Median overall survival (OS) was 12.4 months (95% CI 9.6-15.4) and 8.9 months (95% CI 6.9-9.7) for patients with low (<25) and high (≥25) MII-1 values, respectively (HR = 1.74, 95% CI 1.21-2.51, p = 0.003).
Median OS was 12.6 months (95% CI 9.6-16.2) and 8.9 months (95% CI 6.9-9.7) for patients with low (<1424) and high (≥1424) MII-2 values, respectively (HR = 2.14, 95% CI 1.44-3.20, p = 0.0004).
Median OS was 12.6 months (95% CI 9.8-16.0) and 8.9 months (95% CI 6.9-9.6) for patients with low (<6068) and high (≥6068) MII-3 values, respectively (HR = 1.91, 95% CI 1.98-2.90, p = 0.0005).
Multivariate analysis showed that MII-1, MII-2 and MII-3 were the only independent prognostic factors for OS and they outperformed NLR.
Conclusion: These news immune inflammation indicators represent potential prognostic indicator in patients with advanced HCC treated with sorafenib.

P02-03 Extracellular vesicles derived from CHK2 mRNA as a possible predictive marker of HCC in HCV-infected patients

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Background and aims: HCV infection treatment has experienced a remarkable advancement with the introduction of DAAs. However, HCC recurrence or de novo development in DAA treated patients represents an emerging issue that merits investigation.
A common feature of HCC and dysplastic nodules is represented by chromosomal instability (CIN) that is linked to tumorigenesis. It was recently demonstrated that variation in chromosome structure is an important mechanism of DNA damage, and checkpoint kinase 2 (Chk2) was identified as a central effector of HCC cells response to DNA damage.
It is possible to overcome the several barriers in gathering genetic information in reference to cancer, by capturing and analysing genetic material released from tumour cells into the blood (liquid biopsy). The liquid biopsy test could be valuable to aid with the monitoring of changes in a patient's disease over time.Along these lines, we assessed the diagnostic and prognostic value of CHK2 evaluating its mRNA levels in the liquid biopsies of HCC, and cirrhotic patients as a response to DNA damage and genomic instability with the purpose of locating biomarkers of de novo or recurrent HCC under the screening-detectable size, in patients treated with DAAs.
Method: An Extracellular vesicles fraction was collected from the plasma of patients using the exoRNeasy Serum Plasma Kit and RNA was extracted following Qiagen manufacturer's protocol. Subsequently, CHK2 mRNA levels were evaluated via Real-Time PCR using a TaqMan expression assay and quantified using β-actin and GAPDH as housekeeping genes.
Results: We evaluated extracellular vesicles derived from Chk2 mRNA levels in 15 HCV-HCC patients (10 males, mean age 65 ± 11.7) and 10 HCV-Cirrhotic patients (3 males, mean age 64 ± 12). We found a significant increase in Chk2 mRNA content in extracellular vesicles from infected-HCC and Cirrhotic patients in respect to healthy donors. Moreover, in two patients for which a metachronous sampling was available (before and after HCC occurrence), we observed undetectable levels of CHK2 in the HCC-free plasma sample and levels higher than those of the donors in the HCC-sample.
Conclusion: Our results suggest a possible correlation between CHK2 mRNA and HCC onset.
Furthermore, we found an increase of CHK2 mRNA in 6/10 of the cirrhotic patients suggesting the presence of DNA damage and eventually CIN occurring in hyperplastic/dysplastic nodules. Since dysplastic nodules are classified as precancerous lesions, a strict follow-up could show a relevant early prognostic value of CHK2 mRNA for tumor onset.

P02-04 Clinical characteristics of liver cirrhosis and hepatocellular carcinoma occurring after fontan operation

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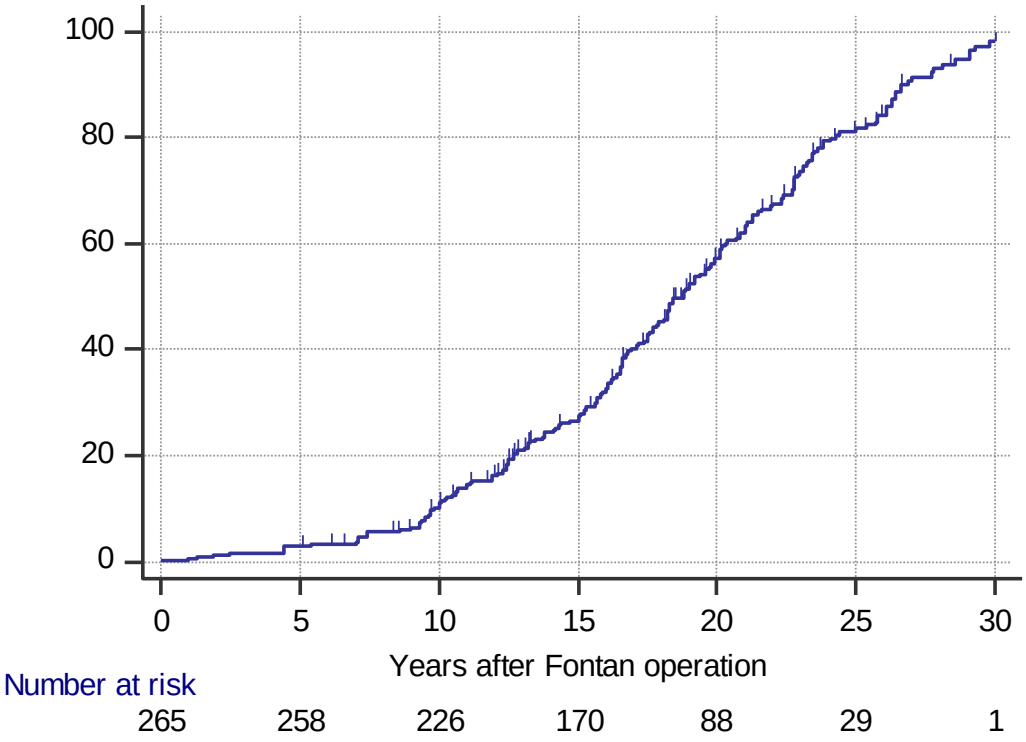
Background and aims: Hepatocellular carcinoma (HCC), which can be arising from cardiac cirrhosis, is one of the most serious late complications in patients after Fontan operation. In post-Fontan patients, benign arterial phase hyper-enhancing (APHE) nodules are frequently observed at multiphasic abdominal CT scan and are difficult to differentiate from HCCs. The aim of our study was to investigate the cumulative incidence of liver cirrhosis (LC), and to identify specific features which can distinguish HCC from benign APHE in patients after Fontan operation.

Method: We performed a retrospective cohort study of patients who had undergone Fontan operation and were followed at our hospital for more than 10 years, and who had undergone ultrasound or CT scan of the liver between January 2000 and December 2015. Cirrhosis was diagnosed using abdominal imaging tests such as ultrasound and CT. The clinical and radiographic features of HCC nodules were compared to those of APHE nodules who had undergone multiphasic abdominal CT scan.

Results: A total of 265 patients with a mean duration after Fontan operation of 19.3 ± 6.6 years were included in the study. The cumulative incidence of cirrhosis at 5, 10, 20, 30 years of duration after Fontan operation were 2.7% (7/265), 13.8% (28/254), 60.5% (135/223), 99.5% (203/204), respectively. The multiphasic abdominal CT scan was performed in 82 patients and APHE nodules were observed in 42 patients. Of the 42 patients, 17 patients had APHE nodules more than 1 cm in size with washout on portal venous and/or delayed phase in cirrhotic liver. In these patients who met current non-invasive imaging diagnosis criteria for HCC, only 6 patients were diagnosed with HCC either by histology (n = 2) or clinical features (n = 4), and therefore, positive predictive value of current non-invasive imaging diagnosis criteria for HCC was only 35.3% (6/17). The presence of washout on portal venous phase ($p = 0.012$) and the serum AFP level ($p < 0.001$) were significantly associated with HCC nodules.

Conclusion: Cirrhosis is a frequently developed late complication in patients after Fontan operation, and its incidence has increased rapidly after 10 years from Fontan operation. Diagnosis of HCC in post-Fontan patients should not be made solely depending on the current imaging criteria, and measurement of serum AFP might be helpful to differentiate HCC nodules from benign APHE nodules.

Figure:


100
80
60
40
20
0
0
5
10
15
20
25
30
Years after Fontan operation
Number at risk
265
258
226
170
88
29
1

P02-05YI Patients with hepatocellular carcinoma (HCC) treated with sorafenib who develop adverse effects during the first 60 days present a characteristic immunologic profile

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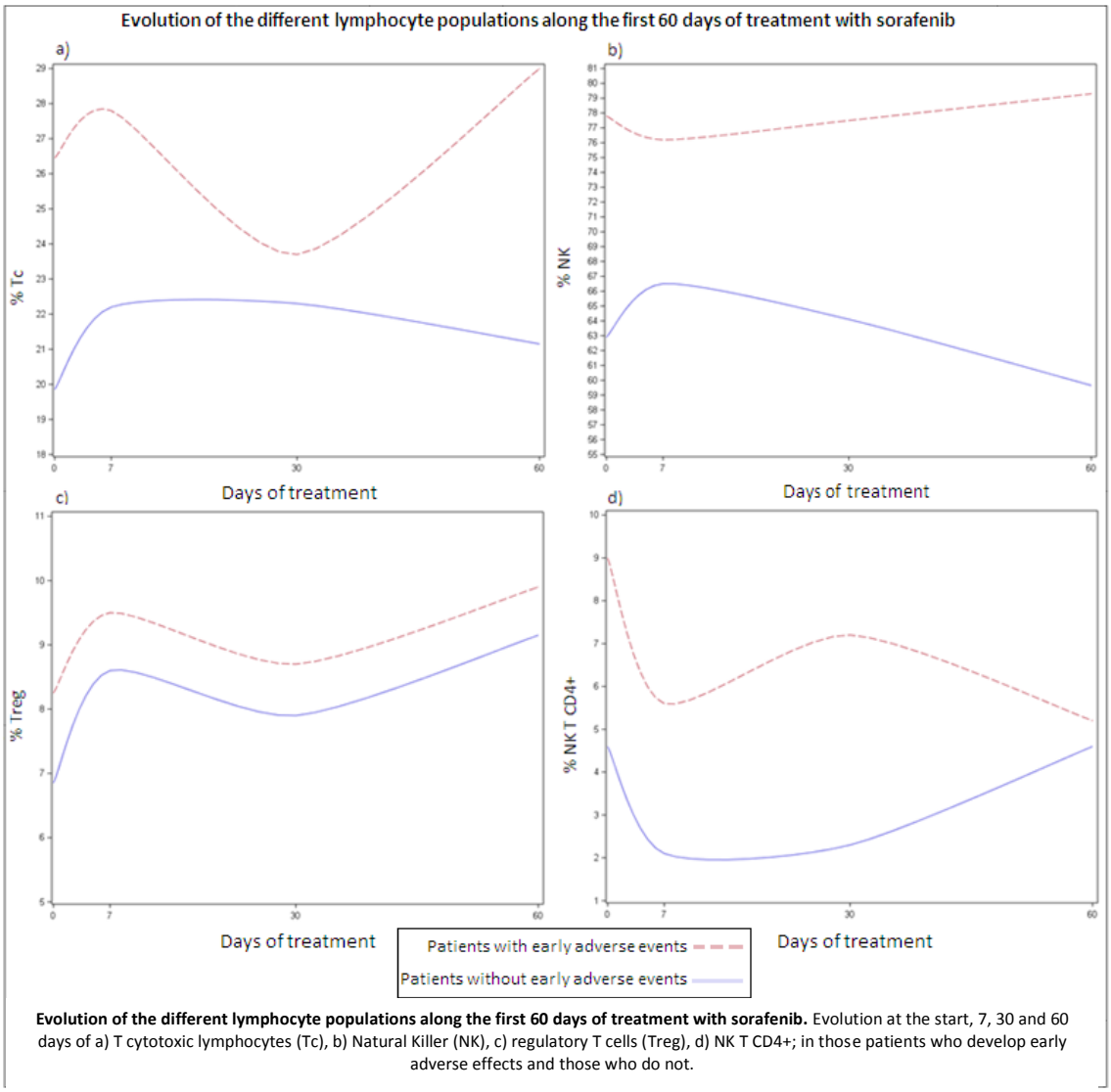
Background and aims: Those patients with HCC treated with sorafenib who develop adverse effects (AE) ≥grade II present better survival (SV). Moreover, the appearance of dermatologic AE along the first 60 days is also a predictor of better SV. In the current study we aim to assess the effects of sorafenib in the lymphocyte populations of the treated patients and the correlation between the lymphocytes evolution and the development of early AE (≤60 days; eAE).
Method: Between 2016-2018, 42 patients started treatment with sorafenib (800mg/day). Blood samples from all patients were collected at the beginning of the treatment, at every month and at every treatment dose modification. The numbers of T lymphocytes, T helper (Th), T cytotoxic (Tc), regulatory T cells (Treg), B lymphocytes, Natural Killer (NK) and NK T were quantified by flow cytometry. We analyzed the medians of each population to assess the progression of the patients' lymphocytes.
Results: Of the 42 patients, 64.3% were BCLC-C, 85.7% were Child-Pugh A and 95.2% were PS-0. Of all patients, 85.7% presented eAE. Before starting the treatment, the levels of Treg (8.25 vs 6.85), Tc (26.45 vs 19.85), NK (77.8 vs 62.9) and NK T CD4+ (9 vs 4.6) were higher in those patients who developed eAE. Interestingly, the NK (29 vs 21.25) and Tc (79.3 vs 59.65) populations experienced an increase along the first 60 days on those patients with eAE but, conversely, decreased in patients without eAE (Fig. a and b). The Treg population showed the same evolutionary pattern in all patients (Fig. c). The NK T CD4+ subpopulation was higher in patients who developed eAE along the first 30 days, the period when most patients (75%) undertake dose modifications; afterwards, it converged at non-eAE patients levels (Fig. d).
Conclusion: Sorafenib has a double effect in the immunologic profile of patients diagnosed with HCC along their first 60 days of treatment. On one hand, those patients who develop eAE show an increase in the cytotoxic populations (Tc and NK) that does not occur in patients without eAE; on the other hand, both groups increase the immunosuppressive lymphocytes by the same level. This suggests that sorafenib induces an immunosuppressing environment which is overruled by the increase in cytotoxic lymphocytes in patients developing eAE.

Figure:



Evolution of the different lymphocyte populations along the first 60 days of treatment with sorafenib. Evolution at the start, 7, 30 and 60 days of a) T cytotoxic lymphocytes (Tc), b) Natural Killer (NK), c) regulatory T cells (Treg), d) NK T CD4+; in those patients who develop early adverse effects and those who do not.

P02-06YI Dissecting the landscape of (epi-)genetic alterations during sequential evolution of liver cancer.

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Background and aims: Development of primary liver cancer is a multi-stage process. Pre-neoplastic dysplastic lesions emerge on the basis of chronic liver damage and evolve into early hepatocellular carcinoma (eHCC) and, subsequently, progressed HCC (pHCC). Detailed molecular characterization and prediction of pre-neoplastic lesions at high risk for malignant transformation would significantly advance our diagnostic and therapeutic approaches. We here utilized integrative molecular analyses to characterize the sequential evolution of liver cancer and aimed to define key epigenetic drivers and biomarkers of HCC development and progression.

Method: Methylation 450k-beadchip analyses were performed on cirrhotic liver (n = 7), low- (n = 4) and high-grade (n = 9) dysplastic lesions, eHCC (n = 5) and pHCC (n = 3) from 8 HCC patients with chronic hepatitis B infection. Differentially methylated gene regions (DMGR) were identified in comparison to non-cirrhotic and non-infected liver (n = 9). Potential epi-drivers and biomarkers were identified by integrative analyses of transcriptomic changes and validated in an independent cohort from the TCGA database.

Results: The proportion of hypermethylated DMGR progressively increased from cirrhosis over dysplastic- to HCC and peaked in eHCC lesions. Early epigenetic alterations involved signaling pathways related to cell death, apoptosis and immune regulation, while late changes centered on cell survival, growth and migration. A common regulation of stem cell-associated pathways including Wnt/b-catenin signaling was revealed in dysplastic as well as eHCC potentially predisposing tumor progression. Moreover, we identified 101 genes with significant methylom changes in dysplastic and cancerous lesions with concomitant progressive gene expression alterations in cancer tissue. We further defined an epi-panel of early epigenetic marks in dysplastic lesions including selected CpG-sites with confirmed differential methylation in cancer tissue and consequential transcriptional alterations of the target genes using an independent cohort of 362 HCC and 49 surrounding liver samples. Unsupervised hierarchical clustering confirmed a robust classification in malignant and non-malignant lesions.

Conclusion: Our results confirm that epigenetic changes occur early during hepatocarcinogenesis. Epigenetic modifications, therefore, might be of high diagnostic/predictive utility for the identification of dysplastic lesions at risk for cancer progression. The identified (epi-)panel of oncogenic epigenetic marks might be useful to complement phenotypic classifications and facilitate selection of lesions amenable to early therapeutic interventions.

Figure:

Figure 1

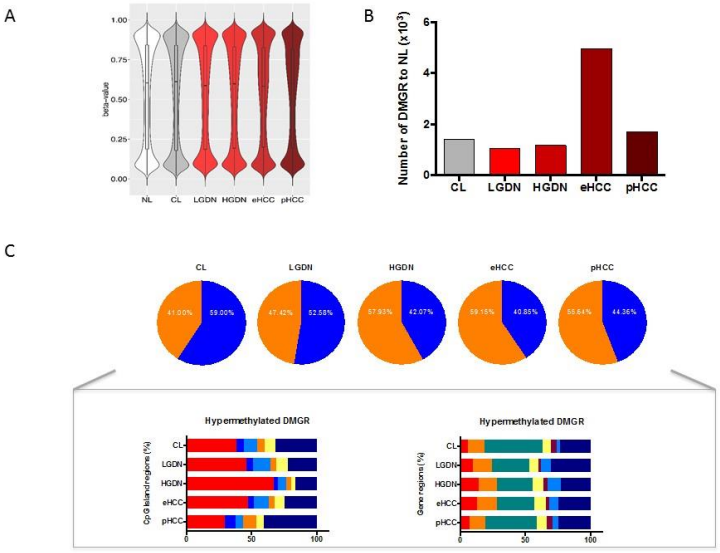
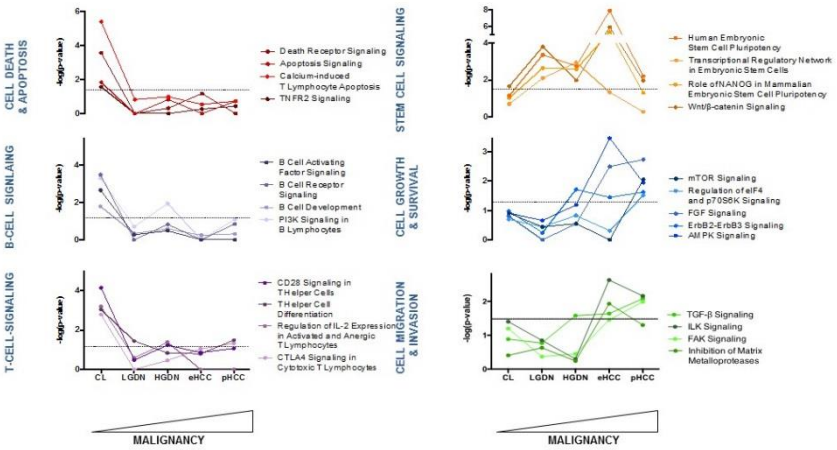


Figure 2



P02-07 Safety, and tolerability abd efficacy of transarterial chemoemebolization using drug elutind microspheres loaded with antracyclines for the treatment of patients with unresectable hepatocellular carcinome : PARIS study?

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Background and aims: Transarterial chemoembolization is currently indicated for the treatment of patients with intermediate-stage hepatocellular carcinoma (HCC) according to the European Association for the Study of Liver Diseases (EASL).

The technology of TACE is constantly improving. A new generation of polyethylene glycol (LifePearl®) microspheres loadable with different anthracyclines, has been developed. The main purpose of PARIS study is to assess safety, liver toxicity and treatment efficacy of LifePearl® microspheres loaded with anthracycline (doxorubicin or idarubicin) for Transarterial Chemoembolization (LP-TACE) in patients with unresectable HCC.

Method: Paris registry is a multicenter, prospective, single arm study of unresectable HCC patients treated with LifePearl® loaded with doxorubicin or idarubicin with safety as a primary end point.

Liver and biliary toxicities were assessed by CT or MRI imaging and by blood tests obtained at several follow-up intervals after LP-TACE treatment. Tumor response was evaluated following hospital standard practice and according to mRECIST criteria and was reported as best overall response (BOR).

Results: Out of 102 enrolled patients 93.14%% were males, with mean age of 65.9 ± 10.4 years and 81.2% with cirrhosis (95.5 % Child Pugh A and 4.5% B). The mean number of HCC lesions was 2.5 ± 1.9 and mean sum of tumor diameters was 70.0 ± 39.0mm.

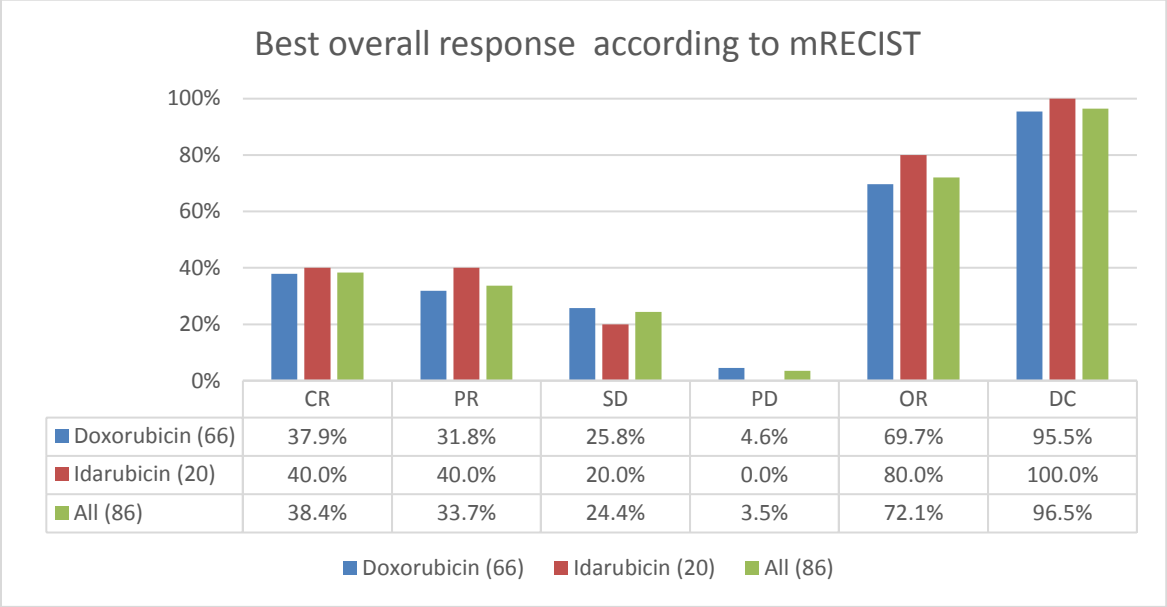
Seventy-five patients (77, 3%) were treated with LifePerarl® loaded with doxorubicin and 22 (22.6%) with LifePearl® loaded with idarubicin with a mean dose of 74.4 ± 22.0mg and 11.7 ± 4.0mg, respectively. A total of 173 LP-TACE procedures were performed.

Adverse events were reported for 58 patients. Serious adverse events were reported in 18 patients, out of which 4 were possibly or probably related to LifePearl®. Different levels of liver/biliary toxicities were detected on follow-up imaging in 17 patients (16.3%), including 3 bilomas.

Objective response (complete + partial response) and disease control rates were 72.1% and 96, 5% respectively in 86 patients for whom at least one imaging control was available. There was no differences between patients treated with doxorubicin or idarubicin as shown in figure 1.

Conclusion: The use of TACE with LifePearl® loaded with doxorubicin or idarubicin for the treatment of patients with unresectable HCC showed good tolerance with relatively low liver/biliary toxicity and very high tumor response rate.

Figure:



	CR	PR	SD	PD	OR	DC
Doxorubicin (66)	37.9%	31.8%	25.8%	4.6%	69.7%	95.5%
Idarubicin (20)	40.0%	40.0%	20.0%	0.0%	80.0%	100.0%
All (86)	38.4%	33.7%	24.4%	3.5%	72.1%	96.5%

Figure 1: Best overall response of patients treated with LifePearl® loaded with doxorubicin or idarubicin. CR (Complete response), PR (Partial response), SD (Stable disease), PD (Progressive disease) OR (Objective response), DC (Disease control)

P02-08 Regulation of miRNA expression by Sorafenib in primary human hepatocytes and liver cancer cells

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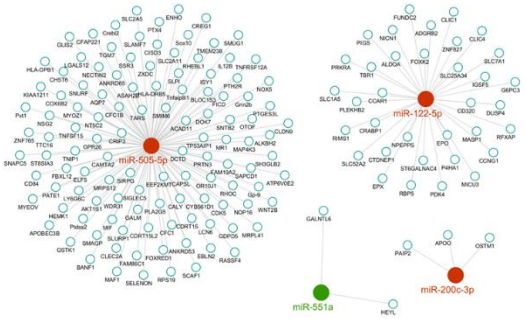
Background and aims: Sorafenib is the first-line molecular therapy for patients in advanced stage of hepatocellular carcinoma. This multikinase inhibitor induces cell cycle arrest and promotes cell death in liver cancer cells. The deregulation of miRNA expression profiles has been associated with tumour initiation and progression, as well as treatment resistance. On this basis, we aimed to determine the relationship between the alteration of miRNA patterns and the induction of autophagy and apoptosis mediated by Sorafenib in liver cancer cells

Method: We carried out a kinetic study of autophagy and apoptosis induction by Sorafenib in HepG2 cells. We have also assessed the effect of the drug in primary cultured human hepatocytes. We determined parameters of cell autophagy (LC3II/LC3I, Beclin-1), apoptosis (caspase-3), migration and invasiveness potential in HepG2 cells. miRNAs were profiled using the using the TaqMan® OpenArray® Human miRNA Panel. Differentially expressed miRNAs were validated and subjected to bioinformatic target prediction with Target Scan database and subsequent enrichment analysis. Eventually, we carried functional studies using with miRNA mimics and inhibitors.

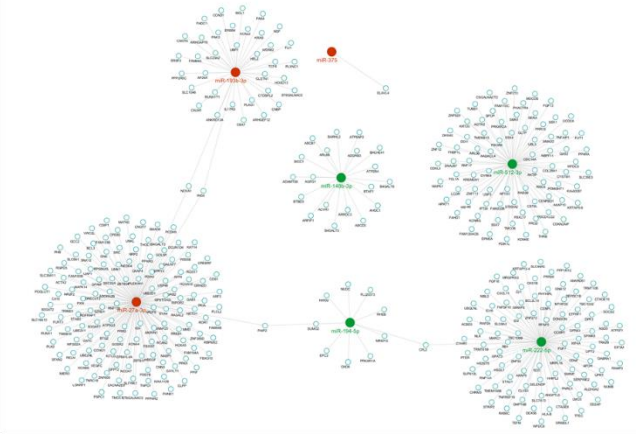
Results: Sorafenib induced transitory autophagy (6 hours) that leads to apoptosis (24 hours). Primary human hepatocytes were greatly resistant to the antiproliferative and pro-apoptotic properties of Sorafenib. The administration of Sorafenib (10 μM) for 6 hours significantly downregulated miR-551a, and upregulated miR-200c-3p, miR-505-5p and miR-122-5p. Target prediction of the miRNA signature detected a total of 157 regulated proteins, being those targets related to allograft rejection and type I diabetes. The treatment with Sorafenib for 24 hours revealed a miRNA signature characterized by a significant downregulation of miR-222-5p, miR-194-5p, miR-148b-3p and miR-512-3p, and upregulation of miR-375, miR-27a-3p and miR-193b-3p. Bioinformatic analysis showed that this expression pattern was associated to MAPK, PI3K, EFG, ERBB2 and Ras signalling pathways as well as gene silencing, carbohydrate metabolism or actin cytoskeleton. Only miR-21-3p was significantly altered in human primary hepatocytes treated with Sorafenib for 24 hours. The functional analysis showed that miRNA differentially altered autophagy and apoptosis, as well as cell migration and invasiveness.

Conclusion: Multiple signalling pathways and cellular processes are responsible for Sorafenib treatment response. In this intricate network of interactions, the miRNA expression profile composed of miR-551a, miR-200c-3p, miR-505-5p, miR-122-5p miR-222-5p, miR-194-5p, miR-148b-3p and miR-512-3p, of miR-375, miR-27a-3p, miR-193b-3p and miR-21-3p has been identified as a signature related to the regulation of cell proliferation, autophagy, cell death, migration and invasiveness.

Figure:



Gene Regulatory Network Sorafenib 6 hours



Gene Regulatory Network Sorafenib 24 hours

P02-09YI NASH as a risk factor for intrahepatic cholangiocarcinoma and its prognostic role: case-control study

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Background and aims: The prevalence of intrahepatic cholangiocarcinoma (ICC) is rising worldwide. The current epidemics of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) might be partly responsible for this trend.

Method: Case control study investigating the prevalence of histology-confirmed NASH in peritumoral liver of resected ICC patients and controls (pre-explant biopsies of liver donors). Controls were matched for age and sex in a 2:1 fashion. Correlates between NASH, tumor characteristics and overall survival (OS) were also explored in the ICC cohort.

Results: Between 2006 and 2017, 84 ICCs were resected in our Institution. Sixty-two (74%) had no apparent risk factors for ICC. Amongst this group, the prevalence of NAFLD and NASH was 45.2% and 24.2%, respectively, compared to 44.3 and 8.9% in the 124 matched liver donors ($p = 1.000$ and $p = 0.007$, respectively). The 5-year OS rate was 20.0% in NASH and 57.4% in ICC without either NASH and other risk factors ($p = 0.017$). Main tumor size, sex and NASH (hazard ratio 2.618, 95% confidence interval 1.140-6.013, $p = 0.023$) were independent predictors of the OS at the multivariate Cox regression.

Conclusion: NASH (but not NAFLD) acts as a risk factor for ICC and may affect its long-term outcome. A collaborative multicenter approach could confirm and strengthen these data.

P02-10YI Dual CCR2/CCR5 antagonism as macrophage targeted therapy in experimental hepatocellular carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) is an inflammation-associated cancer and characterized by specific tumor-associated macrophages (TAMs) which sustain tumor development. The chemokine-receptors CCR2 and CCR5 are involved in attraction and polarization of TAMs. The possibility to target monocyte infiltration and/or their pro-tumoural polarization was investigated by using Cenicriviroc (CVC), a selective dual CCR2/CCR5 antagonist.
Method: HCC was induced in 5-week-old male sv129 mice by weekly diethylnitrosamine (DEN)-injections for 20 weeks. Mice were treated with CVC 100 mg/kg or vehicle by daily oral gavage for 5 weeks (25-30 weeks of age). Liver damage and HCC development were analyzed by liver histology and qPCR, multiplex and flow cytometry analyses on serum and liver tissue. Life CD45+Ly6G-CD11b+F4/80+Ly6C+ infiltrated monocytes, CD45+Ly6G-CD11b+F4/80+Ly6C-Tim4- monocyte-derived macrophages and CD45+Ly6G-CD11bintF4/80+Ly6C-Tim4+ Kupffer cells (KC) were isolated from the liver by fluorescence activated cell sorting (FACS) and analysed for inflammatory and pro-tumorigenic markers by qPCR. The effect of CVC was further investigated in vitro on bone-marrow derived macrophages (BMDM).
Results: CVC treatment was well tolerated and mice showed reduced weight loss compared to vehicle treated mice. Treatment efficacy was confirmed by elevated serum CCL2 levels in CVC treated mice. CVC treatment did not result in a better survival rate or reduced tumor load. However, CVC treated mice showed a tendency to reduced fibrosis, evaluated on sirius red staining, with significant lower expression of MMP9 and MMP12 and the cytokines TNFalpha and IL-10. Flow cytometric analyses did not reveal significant differences between liver monocyte/macrophage populations between CVC and control treated mice while FACS-isolated KCs from CVC-treated mice showed reduced expression of TNFalpha and IL6 and an increased anti-tumoural CD86/CD206 balance compared to KCs from vehicle treated mice. The latter was confirmed in vitro on BMDM were CVC resulted in reduced expression of CD206, Arg-1, iNOS and CCR2.
Conclusion: CVC treatment in DEN-induced HCC did not result in better survival rate or reduced tumor load although the therapy influenced the tumoural micro-environment and might shifted liver KCs towards an anti-tumoural phenotype. Further research with combination therapy seems mandatory.

P02-11 Polyploidy Spectrum: A new marker of HCC tumor classification

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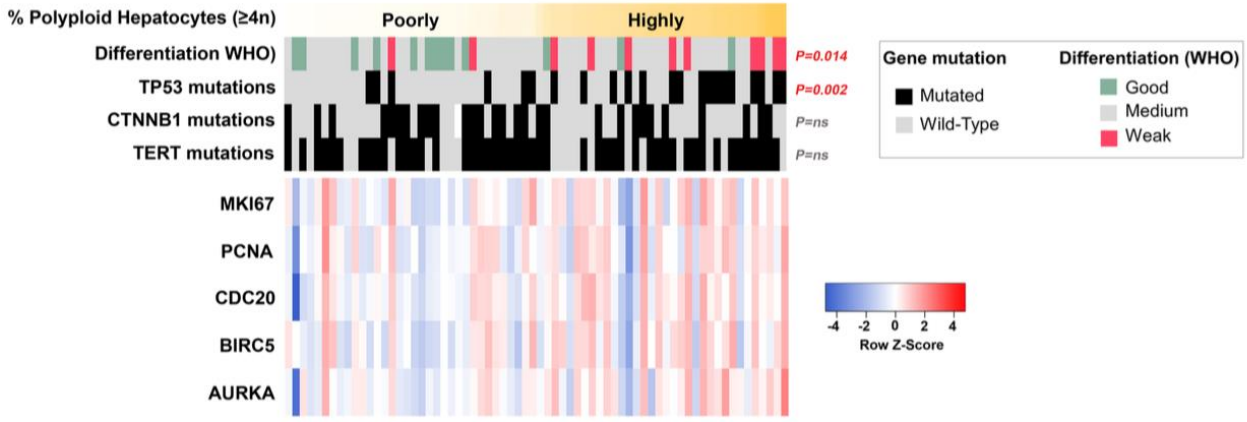
Background and aims: Polyploidy is a fascinating characteristic of liver parenchyma. Hepatocytes polyploidy depends on the DNA content of each nucleus (nuclear ploidy) plus the number of nuclei per cell (cellular ploidy). Which role can be assigned to polyploidy during human HCC development is still an open question. Here, we investigated whether a specific ploidy spectrum is associated with clinical and molecular features of HCC.
Method: Ploidy spectra were determined on surgically resected tissues from HCC patients as well as 5 tissues from healthy control. To define ploidy profiles, quantitative and qualitative *in situ* imaging approach was used on paraffin tissue liver sections.
Results: We first demonstrated that polyploid hepatocytes are major components of human liver parenchyma, polyploidy being mainly cellular (binuclear hepatocytes). Across liver lobules, polyploid hepatocytes do not exhibit a specific zonation pattern. During liver tumorigenesis, cellular ploidy is drastically reduced; binuclear polyploid hepatocytes being barely present in HCC tumors. Remarkably, nuclear ploidy is specifically amplified in HCC tumors. In fact, nuclear ploidy is more amplified in HCCs harboring a low degree of differentiation and *TP53* mutations. Our results finally demonstrated that highly polyploid tumors are associated with a poor prognosis and a higher proliferation rate.
Conclusion: Our results underscore the importance of quantification of cellular and nuclear ploidy spectrums during HCC tumorigenesis.
Figure:



P02-12YI Protein kinase erk5 modulates liver regeneration process in mice

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Background and aims: Hepatic regenerative response is considered a pivotal step during liver tissue repair process upon damage and it is sustained by several cell types and the activation of multiple signaling pathways.
ERK5 is a member of the MAPKs family. We have recently obtained data indicating that ERK5 regulates the aggressive properties of hepatocellular carcinoma cells and is critical for the development and growth of HCC and recently we generated hepatocyte-specific ERK5 knock-out mice (ERK5ΔHep). The aim of this study to investigate the contribution of ERK5 in liver regeneration, through the analysis of the phenotypic response of ERK5ΔHep mice subjected to the experimental model of PH.
Method: The ERK5ΔHep animals were generated crossing the ERK5 floxed mice with mice expressing Cre-recombinase under the control of Albumin promoter. ERK5ΔHep mice animals of 16-24 weeks of age along with their Alb-Cre mice (Control mice) were subjected to a 60-65% partial hepatectomy (PH) and sacrificed at different time ALT and AST in the serum were measured using standard assays. Intrahepatic gene expression was assayed by qPCR.
Results: First we evaluated ERK5 activity during liver regeneration in mice. For this aim, we performed western blot experiments using an antibody specific toward the phosphorylated form of ERK5, which is the activated form of ERK5 and cell lysate obtained from WT livers isolated at different time points. Interestingly phosho-ERK5 signal was found between 6-9 hours after PH, that correspond to the transition from the “priming” phase of liver regeneration where hepatocytes prepare for cell cycle re-entry and the “progression” phase where hepatocyte proliferation is induced.
Control and ERK5ΔHep mice were subjected to PH and sacrificed at 24, 48 and 168 hours. Liver-to body weight ratio showed that liver recovery was similar at 24 and 48 hours after PH whereas at late time point we observed a reduced hepatic regeneration in ERK5ΔHep mice. This finding correlated with reduced hepatic expression cell proliferation markers such as PCNA, cyclin B1 in ERK5ΔHep mice. The reduced liver regeneration observed in ERK5ΔHep mice after PH was accompanied by a severe liver damage at 24 and 48 hours. In fact these animals showed elevated ALT and AST levels and necrotic areas in the liver. In line with these results, at the same time points, we observed increased mRNA levels of Cd14 and Ccl2, two typical markers of monocyte infiltration in ERK5ΔHep livers.
Conclusion: This study provides evidences that ERK5 exerts an important role in liver regeneration process participating to the priming phase that involves the activation of several proinflammatory pathways and to the progression phase that activates the cell cycle machinery of hepatocytes. Further studies are needed to understand the cross-talk between ERK5 and signaling pathways that control hepatic regeneration upon damage.

P02-15YI Hepatocellular carcinoma identification models for patients with hcv-related chronic liver disease using data mining techniques

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Background and aims: The risk of hepatocellular carcinoma (HCC) development increases in parallel with liver fibrosis progression, which causes the need for HCC surveillance for patients with advanced fibrosis. The need for repeating an examination is difficult in some countries due to lack of resources or high cost. Data mining techniques develop an easier, less time to consume, and an accurate and effective approach in clinical decision support for early prediction of HCC. This study aimed to develop prediction models for the diagnosis of chronic Hepatitis C-related HCC using data mining techniques.

Method: A cohort of 3099 patients with chronic hepatitis C infection and a Cohort of 1324 patients with HCV-related HCC were enrolled. Baseline demographic and laboratory data were analyzed to identify the effective parameters for predicting the presence of HCC. Classification models were built using data mining techniques (Classification and Regression tree, Alternating decision tree, reduce pruning error tree and linear regression algorithm) to predict the risk of HCC.

Results: Five parameters (Age, AFP, ALP, albumin, and total bilirubin) were found to be strongly correlated to the presence of HCC. They have P value <0.001 with correlation ≥0.5. These variables were used as independent factors to build the HCC risk prediction models. A cross-validation with 10-fold was used in training all proposed models to avoid the over-training.

The first proposed model was built using multi-linear regression and was produced as:

$$HCC_{identification} = 0.8328 + 0.0084\,\text{Age} + 0.0004\,\text{ALP} + 0.3502 * 10^{-5}\,\text{AFP} - 0.2884\,\text{Albumin} + 0.0705\,\text{Total Biliubin} \quad (1)$$

The second proposed model, an alternating decision tree was learned for the cohort dataset. If the final score of the tree is ≥zero (positive value), the patient is high risked to have HCC. Classification and regression tree (CART) was run in the third model. CART employs 10-fold (default) cross-validation. The reduce error pruning (REP-tree) classifier in the fourth model. The data mining techniques under study were able to predict the presence of HCC in Chronic Hepatitis C patients with AUROC ranging between 95.5% and 99%, and overall accuracy between 93.2% and 95.6%.

Conclusion: Models with simple factors have the ability to identify the HCC development.

Figure:

Technique	Sensitivity S_n %	Specificity S_p %	Positive Predictive Value (PPV)%	Negative Predictive Value (NPV)%	Accuracy %	AUROC %
Linear Regression	89.3	93.9	73.8	97.9	93.2	96.0
ADTree	97.3	91.8	96.5	93.5	95.6	99.0
CART	96.8	87.8	94.9	92.2	94.1	95.6
REP- Tree	97.1	86.3	94.3	92.6	93.8	95.5

P02-16YI Energy metabolism and cell motility/adhesion defect in circulating NK-cells from patients with Hepatocellular Carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) is a complex disease with poor prognosis. Natural Killer (NK) cells play a central role in cell-mediated immune response to cancer. In previous studies number and function of NK-cells have been shown to be positively correlated with HCC outcome. A better understanding of the molecular basis underlying NK-cell function in HCC, may inform on target molecules and cellular pathways to be restored in immunotherapeutic approaches potentiating NK cell response in HCC.
Method: NK cells (CD56+CD3-) were derived by fluorescence-activated cell sorting (FACSAria II) from peripheral blood of 8 patients with Hepatitis C virus (HCV)-related liver cirrhosis, as control, and 15 patients with early stage HCC and HCV-related liver cirrhosis. Gene expression profile was performed by Agilent gene expression microarrays. Differentially expressed genes were defined by GeneSpring, Gene Set Enrichment Analysis (GSEA) and Ingenuity Pathway Analysis (IPA).
Results: Genome-wide expression profiling identified a subset of 5986 genes differentially expressed in NK cells from patient subgroups. Hierarchical clustering and PCA analysis showed partial segregation among groups of patients.
1435 genes were differentially expressed (1114 downregulated) in HCC vs LC. GSEA revealed enrichment and downregulation of genes related to cell metabolism and to cell motility in HCC. Downregulation of pathways involved in NK cell function, cell motility, and energy metabolism was confirme by IPA. Cellular movement and cell-to-cell signaling and interaction functions were predicted to be downregulated in HCC. Molecules associated with cytotoxic response were higher in NK-cells in HCC patients, however NK-cells showed defect in cytokine production and degranulation.
Conclusion: Our results are suggestive of an impairment of cell motility and energy metabolism in peripheral blood NK-cells. These molecular defects may explain reduced cytokine production and degranulation, that may represent one of the mechanisms of tumor progression

P02-17 mtdh/aeg-1/lyric promotes tumor proliferation and targeted drug resistance in hepatocellular carcinoma

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Background and aims: MTDH/AEG-1/LYRIC has been demonstrated as an oncogene overexpressed in almost all solid tumors, suggesting that MTDH might be employed as a universal diagnostic/prognostic marker for cancer. Besides, accumulating evidence indicates that overexpression of MTDH associated with sensitivity of numerous chemotherapy drugs including doxorubicin, 5-fluorouracil, cisplatin, paclitaxel, Mitomycin C and so on, but little studies are about targeted drugs sensitivity. Sorafenib is the first targeted drug that was proven and approved as the first-line drug for advanced Hepatocellular carcinoma. Unfortunately, only a few patients received a long-term benefit from this treatment, largely due to the primary and acquired resistance. The aim of this study was to investigate the molecular mechanism of MTDH in sorafenib resistance and to explore the effect of combination of MTDH inhibitor and sorafenib *in vitro* and *in vivo*.
Method: Cell culture, siRNATransfection, CRISPR-Cas9, Overexpression of MTDH, Apoptosis assay, Western blot, Fluorescence in situ hybridization, RNA Binding Protein Immunoprecipitation, Co-Immunoprecipitation, Luciferase reporter assay, Patient derived xenograft model, Statistical Analysis
Results: MTDH is elevated in HCC patients and sorafenib-resistant HCC cell lines, and it is related to sorafenib sensitivity. Knockdown of MTDH induced apoptosis and promotes Sorafenib sensitivity through targeting downstream protein MAP3K7, whereas overexpression of MTDH promotes tumor proliferation and sorafenib-resistance. CEBPB promotes transcription of MTDH mRNA and forms a positive feedback loop of MTDH/MAP3K7/CEBPB. MTDH negatively regulates FBXW2, an E3 ligase, by binding to its mRNA and decreases the ubiquitination and degradation of MAP3K7. Further experiments *in vivo* are needed.

Figure:

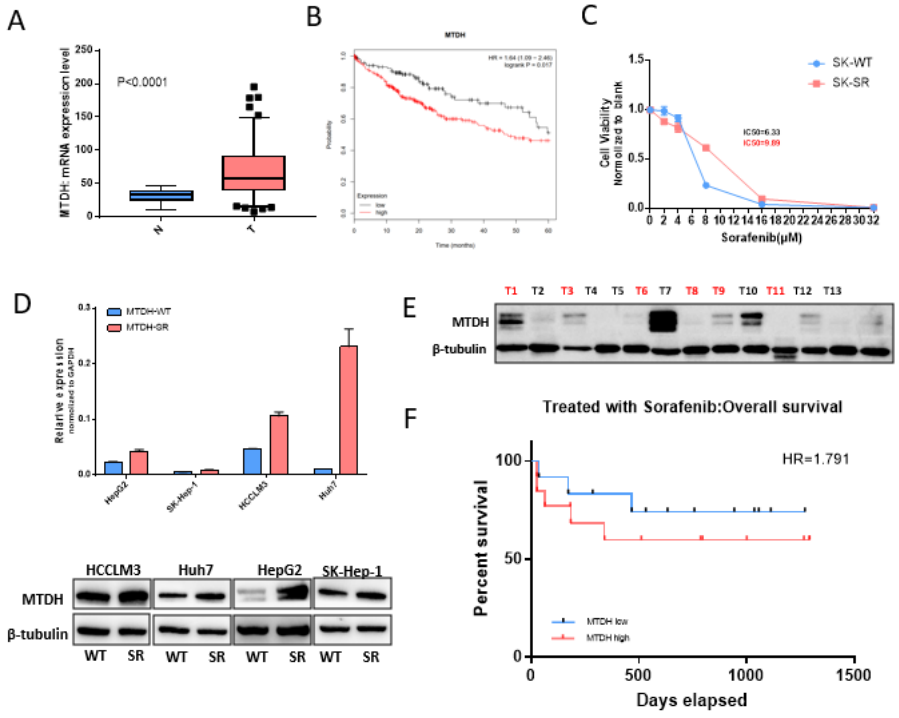

Figure 1. MTDH is elevated in HCC patients and related to sorafenib sensitivity.

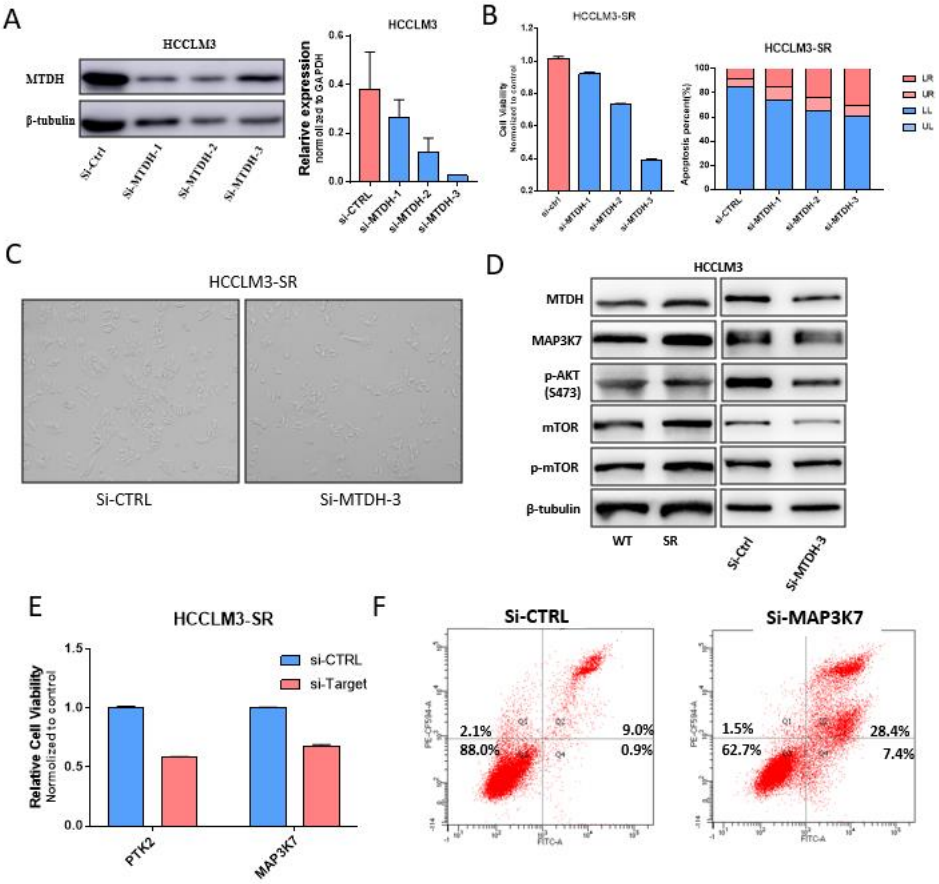

Figure 2. Knockdown of MTDH induced apoptosis and promotes Sorafenib sensitivity through PTK2 and MAP3K7 downstream

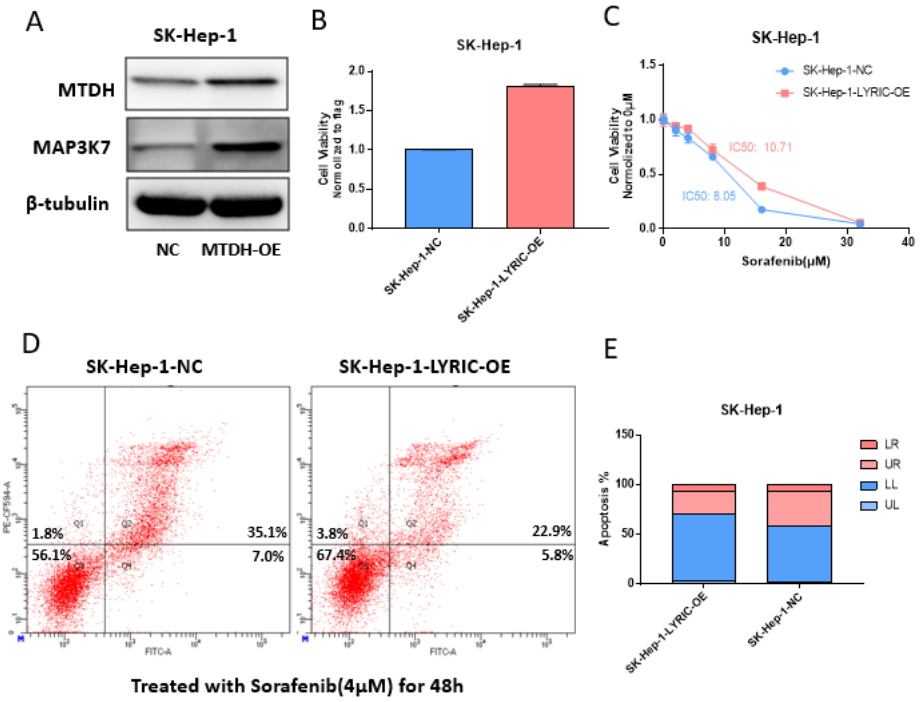

Figure 3.Overexpression of MTDH inhibits apoptosis and promotes sorafenib-resistance through MAP3K7 downstream

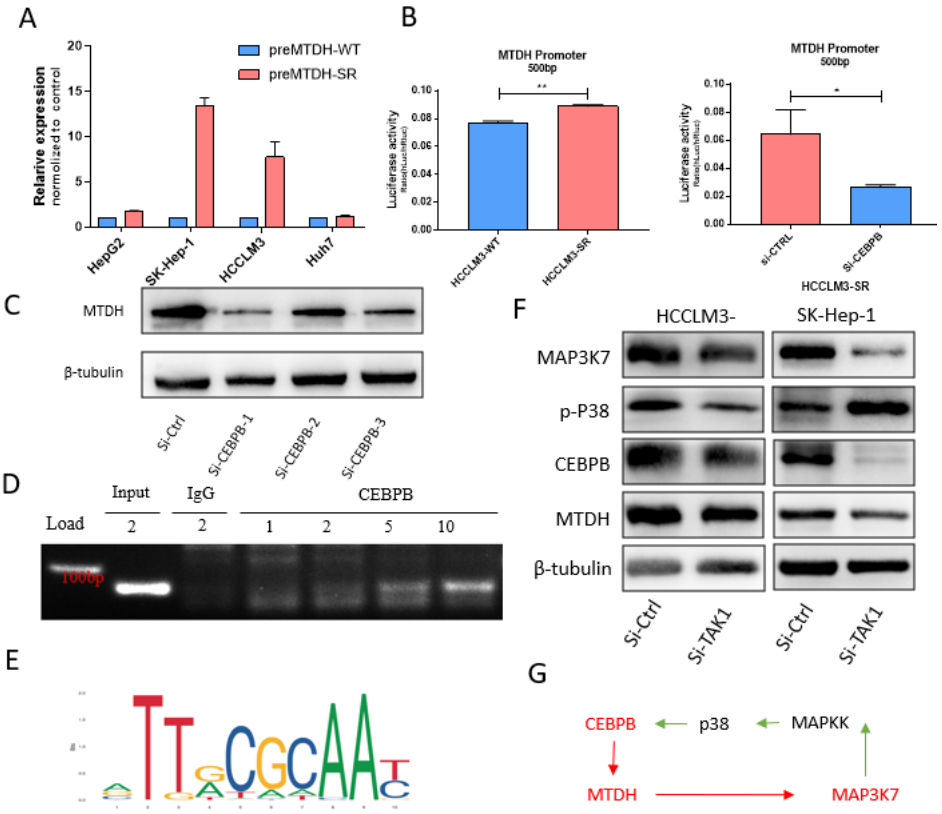

Figure 4. CEBPB promotes transcription of MTDH mRNA and forms a positive feedback loop of MTDH/MAP3K7/CEBPB

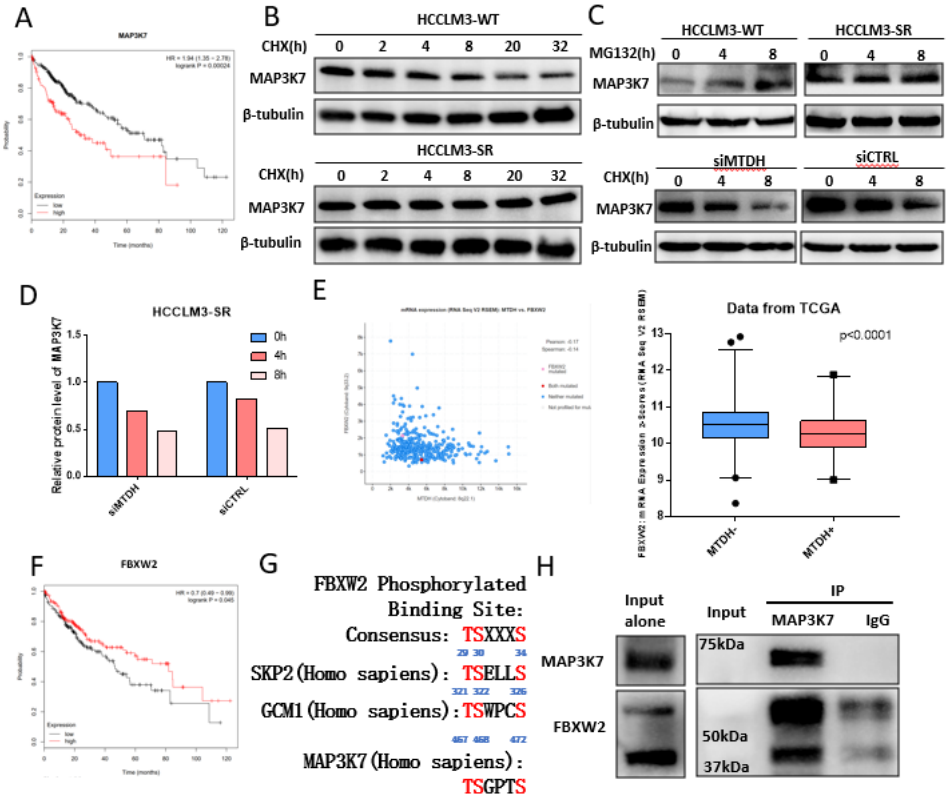

Figure 5.MTDH negatively regulates FBXW2, an E3 ligase, and decreases the degradation of MAP3K7

P03-01YI Efficacy of sorafenib and lenvatinib for hepatitis etiology: a network meta-analysis of phase III trial

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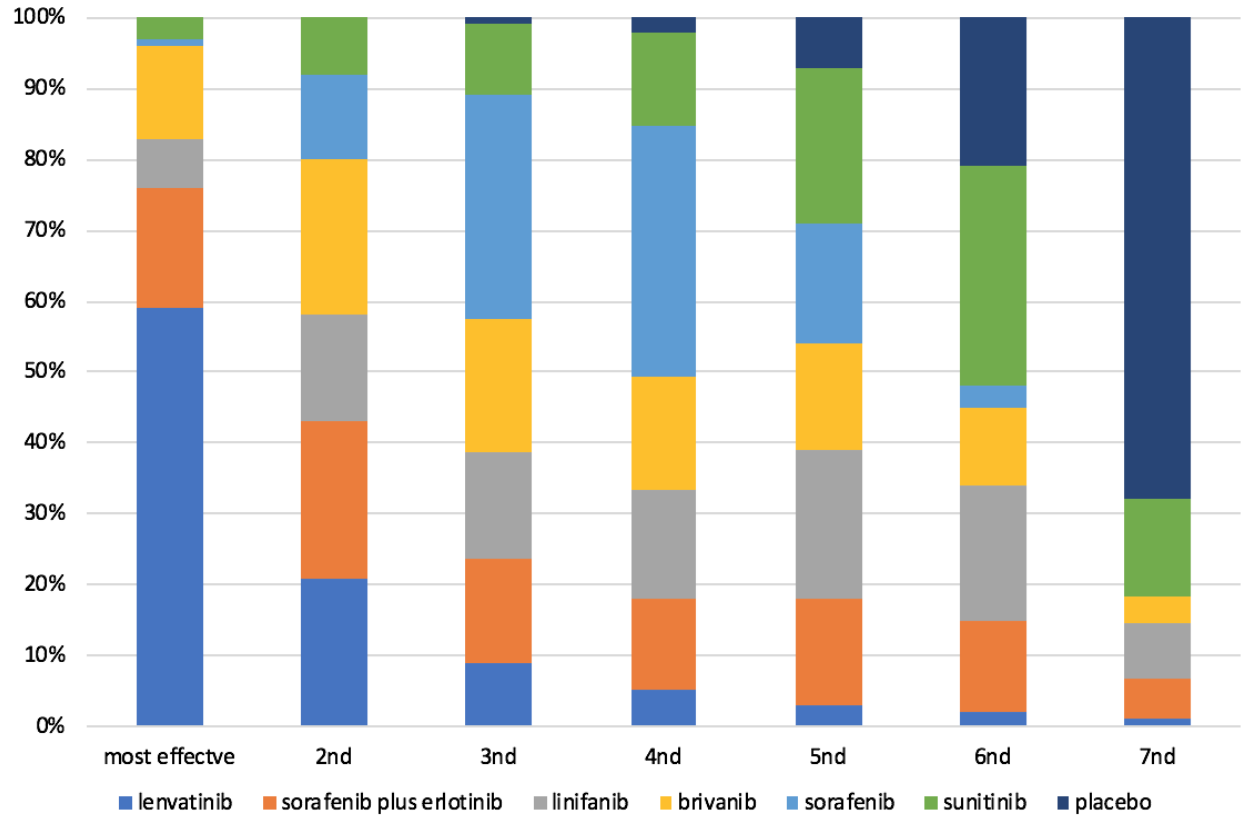
Background and aims: To date, there are no validated prognostic nor predictive markers of response to sorafenib in HCC, although hepatitis status seems to be a potential candidate.
For this reason, we conducted an NMA to evaluate whether the virus etiology could be used to determine which patients may benefit from either lenvatinib or sorafenib.
Method: Data were extracted from the publications or estimated as proposed by Parmar et al.[28]. Treatment effects were estimated by posterior means and 95% credible intervals (CrIs) using random effect, identity link function and non-informative prior distributions (uniform and normal). We performed 25, 000 iterations with burn-in number of 5, 000 iterations and a thin interval of 20 to obtain the posterior distributions of model parameters. Convergence was assessed using the Brooks-Gelman-Rubin method. Posterior distributions were used to assess the probability of each treatment to be the best, second best and so on. Inconsistency and heterogeneity were assessed using node-split models, I2 and Cochran Q tests. Significant heterogeneity was considered to be present for I2 >50% or p value >0.10. Der Simonian and Laird method and random effect were used. All the analyses were made with the R packages "Metaphor" and "Gemtc" (https://www.r-project.org/).
Results: The NMA was performed on a total of 1, 788 patients on six study, of these 1160 patients were HCV-positive or HBV-positive. Of these, 251 (21.6%)HBV-positive patients and 91 (7.8%) HCV-positive patients received lenvatinib, whereas 390 (33.6%)HBV-positive patients and 229 (19.7%) HCV-positive patients received sorafenib.A total of 114 (9.8%)HBV-positive patients and 85 7.3%) HCV-positive patients received placebo.In the overall population no difference was observed between lenvatinib and sorafenib, despite if a slight trend towards a greater efficacy of lenvatinib (HR 0.92, 95% CrI0.61-1.36). Both lenvatinib and sorafenib were significantly better than placebo.
When we restricted the analysis to HBV-positive patients, a significant benefit in terms of OS was estimated for sorafenib (HR 0.78 95% CrI 0.62-0.97) with respect to placebo; for HBV-positive patients there was a clear trend in favor of lenvatinib over sorafenib (HR 0.82 95% CrI 0.60-1.15).
For HCV-positive no differences between lenvatinib and sorafenib were observed (HR 0.91 95% CrI 0.41-2.01). I2, Cocran's Q and node-split models showed no evidence of heterogeneity nor inconsistence, strengthening the results of the NMA.
The rankogram in Fig. 1 reports the probably best approach for these patients. The rankogram show that Lenvatinib was probably the best approach for HBV-positive patients
Conclusion: Our data from NMA highlighted that lenvatinib has a greater activity in HBV-positive patients.

Figure: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sorafenib plus erlotinib
linifanib
brivanib
sorafenib
sunitinib
placebo

P03-02YI Thermal ablation is a safe and effective treatment for intrahepatic cholangiocarcinoma in patients with cirrhosis

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Background and aims: Incidence of intrahepatic colangiocarcinoma (ICC) is increasing worldwide and liver cirrhosis is a well-established risk factor for its development. Surgery is the mainstay treatment, but its applicability is limited in cirrhotic patients. Thermal ablation, including radiofrequency ablation and microwave, is suggested as an alternative treatment, but there are scarce data regarding its efficacy and safety in this population. We aimed to assess the effectiveness, safety and overall survival of thermal ablation as first-line treatment of ICC in patients with cirrhosis.

Method: This is a retrospective analysis of all biopsy-confirmed ICC in cirrhotic patients treated in our unit from 2001 to 2017. Baseline characteristics, ablation procedures and complications were recorded. Time to recurrence (TTR) and overall survival (OS) were calculated using Kaplan Meier method.

Results: Twenty-seven patients were treated in this time period in our unit. 51.7% of patients were men with a median age of 63.7 years. The most frequent cause for cirrhosis was hepatitis C Virus (63%), 70.4% were Child-Pugh A and the majority had clinically significant portal hypertension. None of them had cancer-related symptoms (ECOG-PS 0) and tumor markers (CEA, AFP and CA 19.9) were not elevated. Median tumor size was 21 mm [IQR 20-28 mm], 21 cases were uninodular (stage Ia AJCC 8th edition) and 6 were at stage II. Finally, among those patients with single ICC, 10 of them had a single $\leq$2cm ICC.

Complete response was achieved in 25 cases (92.6%). Median OS of the whole cohort was 30.6 months (CI 95%; 22.6-46.5), and recurrence was detected in 21 cases (77.8%) with a TTR of 10.1 months (CI 95%; 7.7-20.9 months). In those patients with single $\leq$2cm ICC, the OS was 94.5 months (CI 95%; 11.7-not reached) and this OS is statistically superior to those patients with single ICC larger than 2 cm (24.3 months (CI 95%; 10.4-44.25 months), p = 0.04) and to those with multinodular disease (26.5 months (CI 95%; 20.23-41.4); p = 0.02). Regarding safety in the whole cohort, only two patients presented a treatment related complication.

Conclusion: Thermal ablation is a safe and effective alternative to surgery for ICC in patients with cirrhosis. The global OS is similar to the reported in surgical series, but the initial treatment success is hampered by a high rate tumor recurrence. Long-term survival after thermal ablation is achieved in patients with single $\leq$2cm ICC and is statistically superior compared to single ICC >2 cm or intrahepatic multinodular ICC.

P03-03YI A novel versatile murine cholangiocarcinoma model via CRISPR/Cas9-mediated sequential oncogenic mutations of liver organoids

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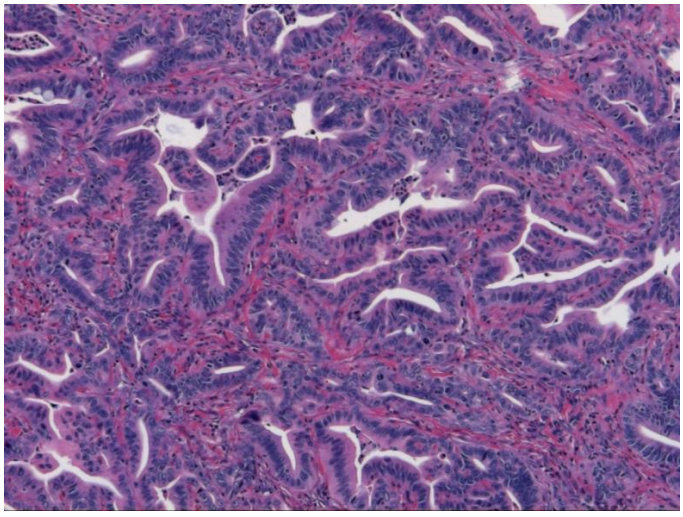
Background and aims: Cholangiocarcinoma retains high mortality rates due to limited treatment options. Recent cancer genomic analyses have identified numerous genetic mutations in CCA, which require further validation to explore their contribution to the disease and therapeutic targeting potential. Animal modeling of CCA is a critical tool to functionally study the cancer biology in its entire complexity, but the use of current genetically modified animals is limited by lengthy generation times with restricted genetic mutations, and patient derived xenografts require an immunosuppressed environment. We hypothesized that by genetically editing wildtype liver organoid cells, which feature a stem-, and biliary-like phenotype, we will create a genetically versatile and modifiable, syngenic modeling system for CCA.
Method: Murine primary liver organoids were generated, and oncogenic mutations introduced with transfection of a plasmid harboring SpCas9 and specific sgRNAs to sequentially disrupt *Tp53* (P), *Kras* (K) and *Smad4* (S). An oncogenic *Kras*^{G12D} mutation was introduced with a mutated donor template. Mutated clones were isolated by functional selection. A fluorescent reporter line for transcriptional activity of YAP, an oncogenic transcriptional activator upregulated in the majority of CCA, was generated by lentiviral integration. 10^6 triple-mutant PKS organoid cells were injected subcutaneously into syngenic mice, and tumors analyzed by histology.
Results: Liver organoids showed increased proliferation rates with concordant loss of growth factor dependence upon each additional mutation but retained their spherical morphology. Subcutaneous transplantation of PKS organoids into syngenic hosts led to tumor formation with 100% penetrance (n = 15) within 4 weeks. Histological analysis revealed a morphology strikingly similar to human CCA specimens with glandular growth pattern (Figure, HandE stain), expression of biliary markers and extensive recruitment of stromal and immune cells from the host. YAP transcriptional activity by fluorescence was heterogenous and strongly accentuated in cancer cells at the tumor border.
Conclusion: This proof-of-principle study demonstrates successful CCA formation with triple-mutated liver organoids including the feasibility for functional assessment, exemplified with heterogenous expression of a YAP activity reporter, thus introducing a novel CCA *in vivo* modeling approach that allows for fast and expandable genetic modifications and versatile hypothesis-driven applications.
Figure:



P03-04YI Liver polyploidization during NAFLD: a Gatekeeper against Replication Stress

Romain DONNE[1], Maeva Saroul[1], Mounia Herrag[2], Patrick Revy[2], Philippe Gual[3], Valérie Paradis[4], Chantal Desdouets[1]
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Background: Over the past decades, the rising incidence of HepatoCellular Carcinoma (HCC) has paralleled to the increase prevalence of obesity. The liver is a central organ affected by high level of fat accumulation, defined as Non-Alcoholic Fatty Liver Diseases (NAFLD). Alarmingly, hepatic steatosis (NAFL) combined with chronic inflammation and liver injuries cause Non-Alcoholic Steatohepatitis (NASH), the more severe form of NAFLD and a precursor of HCC. Increasing evidence indicates that NAFLD strongly affects the intrinsic proliferative properties of hepatocytes. Several signs of impaired proliferation status were reported in fatty hepatocytes such as telomeres attrition and senescence's engagement. Recently, our team demonstrated that fatty hepatocytes divide preferentially by endoreplication (skip mitosis) due to the activation of the **D**NA **D**amage **R**esponse (DDR). Endoreplication is considered as an alternative division program in a context of genomic stress and leads to the genesis of polyploid contingents.
Methods and Results: We developed a powerful quantitative and qualitative technological approach to define ploidy profiles on tissue liver sections. We demonstrated a dramatic enrichment of highly polyploid hepatocytes (≥8n) in NASH murine models (**H**igh-**F**at **H**igh-**S**ucrose/**C**holine-**D**eficient **H**igh-**F**at **D**iets) but also in NASH human patients. Importantly, this population is barely seen in normal condition. Within fatty parenchyma, polyploid fraction co-localize with lipid droplets suggesting an adaptation process to store and/or metabolize lipids more efficiently. We go further to decipher why the DDR is activated in fatty parenchyma leading to the genesis of the polyploid fraction. Transcriptomic analysis reveals an enriched gene set involved in DNA repair in fatty hepatocytes. In this context, DNA synthesis parameters were measured in dividing primary hepatocytes cultures (HFHS/CTR). DNA combing assay reveals that NAFL hepatocytes exhibit a dramatic reduction of replication fork speed with the presence of stalled fork. Regarding the presence of DNA breaks by Comet assay, replicating NAFL hepatocytes display high level of breaks associated with a pan-nuclear of gamma-H2AX staining. Additional molecular analyses reveal a specific activation of ATR/pRPA^{S33} pathway, specifically activated by single-strand DNA Breaks. Interestingly, double-strand DNA Breaks are found in replicating NASH hepatocytes.
Conclusion: Collectively, our results showed that dividing fatty hepatocytes harbor Replication Stress; RS being recognized as a hallmark of cancer. We suggest that the genesis of polyploid hepatocytes could buffer these DNA lesions and thus represent a defense mechanism in NASH-HCC sequence.

P03-05 Radioembolization with Yttrium-90 microspheres for intermediate-advanced hepatocellular carcinoma: a single centre real-life experience

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Background and aims: Hepatocellular carcinoma (HCC) is a common cause of worldwide mortality. In the last years, transarterial radioembolization (TARE) with Yttrium-90 microspheres has been proposed for both intermediate HCC poorly responsive to transarterial chemoembolization (TACE) and locally advanced HCC with segmental or lobar portal vein thrombosis (PVT). The study aim was to evaluate efficacy and safety of TARE in HCC patients. A secondary aim was to confirm the treatment utility in special situation, i.e. tumors complicated by PVT and bridge to liver transplant.
Method: From November 2015 to April 2018 20 consecutive patients (M = 19, F = 1, median age 71 [24-85] years) with HCC were selected for TARE by the multidisciplinary HCC board. TARE was performed using 90Y glass microspheres. Tumor response was assessed using computed tomography or magnetic resonance at 3 months evaluated according to modified Response Evaluation Criteria in Solid Tumor.
Results: Among 20 patients treated with TARE, 1 (5%) had early, 10 (50%) intermediate and 9 (45%) advanced stage HCC. HCCs were firstly diagnosed 18 (range 2-96) months before TARE. All patients except three underwent previous treatments for HCC (median number of treatments 2, range 0-6). Other baseline features were: aetiology: hepatitis virus in 7 (35%), alcohol in 7 (35%), multiple in 6 (30%); Child-Pugh class A in 17 (85%), B7 in 3 (15%); median MELD score 7.5 (IQR 6.5-8); pathologic type: unifocal in 7 (35%), multifocal in 13 (65%); PVT in 9 (45%); comorbidities in 17 (85%); performance status: 0 in 7 (35%), 1 in 13 (65%). Only one patient presented side effects: ascites easily controlled with medications. Imaging evaluation at 3 months was performed in 19 patients. Complete response occurred in 13 (65%), partial response in 3 (15%), while progression was seen only in 3 cases (15%). During a median clinical follow-up of 13 months 2 patients repeated TARE due to partial response, 4 underwent TACE and 1 radiofrequency thermoablation due to recurrence of HCC and 2 patients underwent liver transplantation after successful down-staging. Death occurred in 10 patients: 7 died of hepatic failure due to HCC progression, the remaining 3 died of hepatorenal syndrome, heart failure and gastrointestinal bleeding. Median progression free survival and overall survival were respectively 12 and 16 months. HCC patients with or without PVT had no difference in overall survival (median overall survival 14 months in patients with PVT and 25 months in patients without PVT, log-rank test p = 0.33).
Conclusion: TARE was a valid and safe treatment option in patients with intermediate-advanced HCC stages even in the presence of PVT. Good median survival and no severe adverse effects were seen in this group. TARE was a suitable procedure for elderly comorbid patients and also led to down-staging, potentially allowing liver transplantation.

P03-06YI Globally relevant models for hepatocellular carcinoma survival applicable before and after TACE

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[1]National Liver Institute, Hepatology and Gastroenterology, Shebine Elkom, Egypt, *[2]University of Heidelberg, Department of Medicine Salem Medical Center and Center for Alcohol Research, Heidelberg, Germany*, *[3] Amsterdam University Medical Centers, Department Gastroenterology and Hepatology, Amsterdam, Netherlands*, *[4]University of Nottingham, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom*, *[5]Institute of Digestive Disease, Xijing Hospital, Fourth Military Medical University, Xijing Hospital, Xi'an, China*, *[6] Ogaki Municipal Hospital, Department of Gastroenterology, Ogaki, Japan*, *[7]The Chinese University of Hong Kong, Department of Clinical Oncology, Hong Kong, Hong Kong*, *[8]Prince of Wales Hospital, Department of Clinical Oncology, Hong Kong, Hong Kong*, *[9] UCL Cancer Institute, University College London, Department of Oncology, Royal Free Hospital, London, United Kingdom*, *[10]Aintree University Hospital, Department of Hepato-Biliary Medicine, Liverpool, United Kingdom*, *[11] University of Liverpool, Department of Biostatistics, Liverpool, [12]Medical Center University of Freiburg, Department of Medicine II, Freiburg, Germany*, *[13]Hannover Medical School, Department of Gastroenterology, Hepatology and Endocrinology, Hannover, Germany*, *[14] S. Orsola-Malpighi University Hospital, Radiology Unit, Department of Diagnostic and Preventive Medicine, Bologna, Italy*, *[15] Morgagni-Pierantoni Hospital. Alma Mater Studiorum- University of Bologna, Department of Medical and Surgical Sciences-DIMEC, Bologna, Italy*, *[16] Medical University of Vienna, Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Vienna, Austria*, *[17]Clinica Universidad de Navarra, CIBEREHD, Pamplona, Spain*, *[18] National Liver Institute, Menoufia University, Hepatology and Gastroenterology, Shebine Elkom, Egypt*, *[19]University of Liverpool, Department of Molecular and Clinical Cancer Medicine, Liverpool, United Kingdom*

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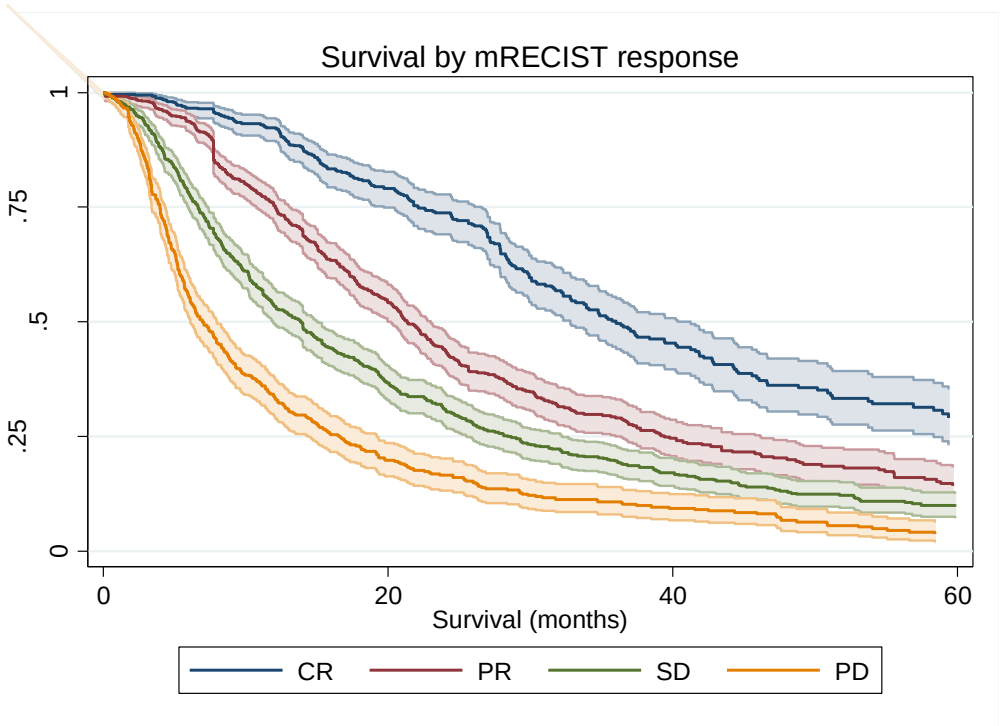
Background and aims: The heterogeneity of intermediate stage HCC and the widespread use of transarterial chemoembolization (TACE) outside recommended guidelines has encouraged the development of scoring systems (e.g. (HAP)[1] and modified mHAP-III[2] scores) that can predict survival after TACE. We aimed to build an internationally validated model by refining existing prognostic scores, offering individualised patient prediction before and after first TACE, and include vascular invasion, aetiology, and response to TACE.

Methods: Patients were recruited from 19 centres representing 11 different countries. Globally available and clinically relevant baseline parameters and radiological response (as assessed by mRECIST) were collected. For model development, we divided the dataset in 3 subgroups (training, internal and external validation sets). Three multivariable Cox regression models were built to discriminate risk groups. The model performance in predicting overall survival (OS) was measured using Harrell's C-index and compared with existing models.

Results: Median OS of all patients (n = 4621) was 19.9 months (95% CI 19.1-20.7 months). The dataset was divided in a training set (n = 1714), internal validation set (n = 1714) and an external validation set (n = 1193). Model 1 confirmed the prognostic influence of the variables in the mHAP-III model. Model 2 showed that adding HCC aetiology and macrovascular invasion (MVI) improved the fit of mHAP-III. Adding radiological response after first TACE further improved the predictive performance (Model 3). Response was an independent prognostic factor for OS (Figure). Four distinct risk groups were observed in each of the validation subgroups with a median OS of the risk groups ranging from 36-44 months in the good risk group (1) to 5-7 months in the poorest risk group (4).

Conclusion: Adding aetiology, MVI and radiological response after first TACE improves the performance of currently available predictive models. This refined model is globally validated and offers personalised survival prediction.

Figure 1. Overall survival according to mRECIST response.



P03-07YI Non-parenchymal TREM2 halts hepatocarcinogenesis through the inhibition of liver inflammation and hepatocyte proliferation

Aitor Esparza-Baquer[1], Ibone Labiano[1], Omar Sharif[2 3], Fiona Oakley[4], Pedro Miguel Rodrigues[1], Elizabeth Hijona[1 5], Raul Jimenez-Aguero[1], Adelaida La Casta[1], Marco Youssef William Zaki[4], Colm O Rourke[6], Patricia Munoz-Garrido[6], Jesper Andersen[6], Sylvia Knapp[2 3], Derek A Mann[4], Luis Bujanda[1 5], Jesús María Banales[1 5 7], María Jesús Perugorria[1 5 7]

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Background and aims: Hepatocellular carcinoma (HCC) is a prevalent and aggressive cancer that usually arises on a background of chronic liver injury where liver regenerative and inflammatory processes are involved. The triggering receptor expressed on myeloid cells 2 (TREM2) is predominantly expressed in hepatic non-parenchymal cells and inhibits Toll-like receptor (TLR)-derived signalling, protecting the liver from various types of hepatotoxic injury. However, its role in liver cancer is unknown. Here, the role of TREM2 in hepatocarcinogenesis and liver regeneration was investigated.

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

Results: *TREM2* expression was induced in human HCC tissue compared to normal liver tissue. In addition, *Trem2* expression was upregulated in the livers of DEN-induced carcinogenic liver injury mouse model as well as during liver regeneration after partial hepatectomy (PHx). *Trem2*$^{-/-}$ mice developed more liver tumours irrespective of size after diethylnitrosamine (DEN) administration, displayed exacerbated liver damage, inflammation, oxidative stress and hepatocyte proliferation. Notably, administration of an anti-inflammatory diet blocked DEN-induced hepatocarcinogenesis in *Trem2*$^{-/-}$ mice. Moreover, *Trem2*$^{-/-}$ livers showed increased hepatocyte proliferation and inflammation after partial hepatectomy (PHx). Supernatant from human hepatic stellate cells that overexpress TREM2 inhibits human HCC spheroid growth *in vitro*.

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

P03-08YI To cure or not to cure intermediate stage hepatocellular carcinoma beyond Milan Criteria: trans-arterial chemoembolization versus surgery in a multicentric matched cohort

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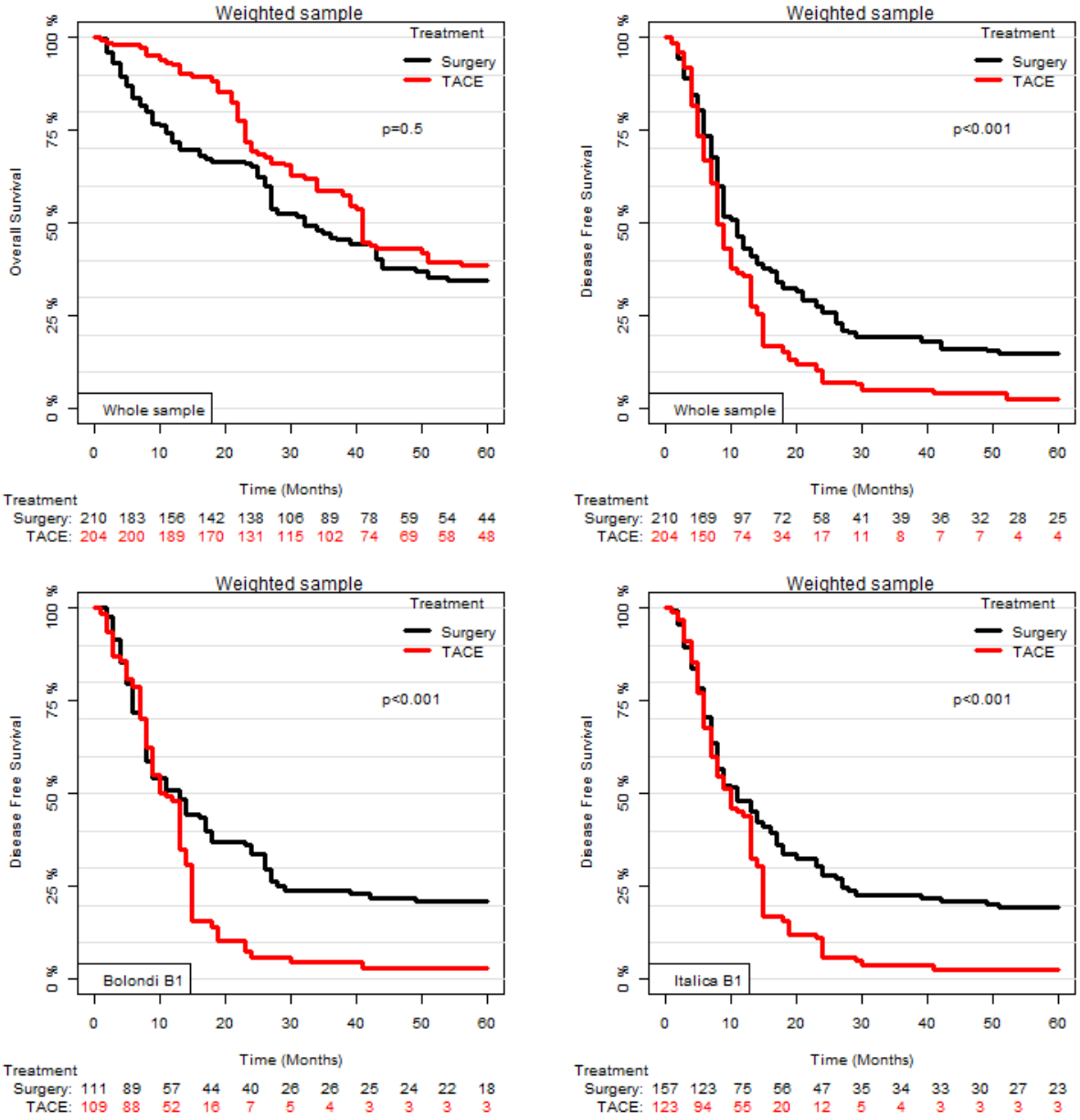
Background and aims: It is still debated if hepatocarcinoma (HCC) beyond Milan Criteria (MC) may benefit the more from curative or palliative treatments. The aim was to assess the overall-survival (OS) and disease-free-survival (DFS) for intermediate HCC beyond MC when treated by trans-arterial-chemoembolization (TACE) versus partial hepatectomy (PH). Survival was assessed even after sub-classification according to Bolondi and ITA.LI.CA. proposals.

Method: between 2008 and 2015, all consecutive patients with a first diagnosis of HCC beyond MC (1 nodule >5cm, or 3 nodules >3cm) were evaluated. Patients were divided according to the assigned treatment. Analysis was carried out through Kaplan Meier curves and Cox regression models. Inverse Probability Weighting (IPW) was adopted to reduce selection bias. Sub-analyses were performed after tumors sub-classifications according to Bolondi (B1, B2, B3, B4) and ITALICA (IB1, IB2, IB3) proposals.

Results: 226 consecutive patients were enrolled: 118 in PH group and 108 in TACE group. The latter were more often cirrhotic (90.7% vs 74.6% in PH, p:0.002), with a median of 3 (IQR 2- 4) nodules (versus 1, IQR 1-2, p:<0.001) and a median size of 4 cm (IQR 3.3-5.5) versus 6 cm (IQR 5-8.8, p:<0.001). After IPW, the two pseudo-populations were comparable for tumor burden and liver function. Median OS was 41 months (95%CI: 39-50) for TACE, while it was 32 months (95%CI: 27- 43) for PH (p: 0.50). Median DFS was 8 months (95%CI: 8- 9) for TACE, while it was 11 months (95%CI: 9- 12) for PH (p <0.001). Being a Child-B (HR 1.8; 95%CI: 1.1-2.8; p: 0.007) and being treated by TACE (HR 1.5; 95%CI: 1.1-2.1; p: 0.015) were independent predictors of recurrence. In B1 group (Child Score 5-6-7, beyond Milan Criteria but inside the Up-to-7), median DFS was 13 months (95%CI: 8-17) for PH, while it was 11 months (95%CI: 9-13) for TACE (p <0.001). Same advantage was evident in IB1 (1 nodule >5cm or 2-3 nodules between 3 and 5 cm) group (PH: 11 months, 95%CI: 6-27; TACE: 10 months, 95%CI: 6-15; p <0.001).

Conclusion: Intermediate stage HCC beyond MC is an heterogeneous group in which OS remains poor. However, surgery may offer a slight but significant better control of the disease, particularly in case of well-compensated liver function and a favorable tumor burden as when tumors are within Bolondi B1 or ITALICA B1 sub-stages.

Figure:


Weighted sample
Treatment
Surgery
TACE
p=0.5
Overall Survival
100 %
75 %
50 %
25 %
0 %
Whole sample
0
10
20
30
40
50
60
Time (Months)
Treatment
Surgery: 210 183 156 142 138 106 89 78 59 54 44
TACE: 204 200 189 170 131 115 102 74 69 58 48
Weighted sample
Treatment
Surgery
TACE
p<0.001
Disease Free Survival
Whole sample
Time (Months)
Treatment
Surgery: 210 169 97 72 58 41 39 36 32 28 25
TACE: 204 150 74 34 17 11 8 7 7 4 4
Weighted sample
Treatment
Surgery
TACE
p<0.001
Disease Free Survival
Bolondi B1
Time (Months)
Treatment
Surgery: 111 89 57 44 40 26 26 25 24 22 18
TACE: 109 88 52 16 7 5 4 3 3 3 3
Weighted sample
Treatment
Surgery
TACE
p<0.001
Disease Free Survival
Italica B1
Time (Months)
Treatment
Surgery: 157 123 75 56 47 35 34 33 30 27 23
TACE: 123 94 55 20 12 5 4 3 3 3 3

P03-09YI Comparison between rates of hepatitis c-related hepatocellular carcinoma recurrence in patients who received or not direct acting antiviral treatment

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Background and aims: Direct acting antiviral agents (DAAs) achieved higher sustained virologic response (SVR) rates and less side effects in a shorter time frame than combined interferon and ribavirin therapy. There is a debate regarding an increased rate of de novo and recurrent HCC in patients with HCV-related cirrhosis who were treated with DAAs in the different populations. The aim of the study is to report the rate of recurrence of HCC following DAAs treatment in an Egyptian cohort of patients where HCV genotype-4 is the most prevalent.
Method: The present study included 333 cases with HCV-related HCC who attended the HCC multidisciplinary clinic, Cairo University from February 2012 till February 2017. All patients had a primary HCC tumor that was treated and showed complete response according to the modified RECIST criteria. Patients were divided into: group 1: 60 patients who received DAAs following complete ablation of their primary tumor, group 2: 273 patients who did not receive DAAs. DAA regimens were decided for each patient according the chronological changes in the recommendations of the Egyptian national committee for control of viral hepatitis.
Results: There was no significant difference between the two groups regarding the patients' demographic, performance status, laboratory characteristics or the site, size and number of hepatic focal lesions. There was no significant statistical difference between the two groups regarding the modality of primary HCC ablation. The rate of HCC recurrence was higher in group (1) 27/60 (45%) in comparison with group (2) 52/273 (19%) showing highly significant statistical difference between the two groups with P value <0.001. The mean survival duration of patients in group (1) was 34.23 months with S.D. ± 16.16months this is significantly higher than the mean duration of survival of patients in group (2) which was 23.92months with S.D. + 13.99 months with significant p value <0.001. Multi-variant regressive meta-analysis was done for the studied patients given DAA after HCC ablation, to predict the most important risk factor that influence HCC recurrence, showed that the size of HCC focal lesion is the predictor of HCC recurrence with p value 0.008.
Conclusion: the rate of recurrence of HCC following DAAs treatment was higher than in those patients who did not receive DAAs with higher survival time. Tumor size was the independent prognostic factor of recurrence.
Figure:

Treatment modality	Group 1		Group 2		
	Patients (60)		Control (273)		P value
	Count	%	Count	%	
Combined	1	1.7	11	4.0	0.913
MWA	19	32.2	80	29.3	
PEI	4	6.6	7	2.5	
RFA	3	5.1	10	3.7	
Surgery	1	1.7	7	2.6	
TACE	35	59.3	165	60.4	
Rate of HCC Recurrence	27	45.0	52	19	<0.001
Survival (m)	34.23	16.16	23.92	13.99	<0.001

P03-10 Interventional targeting of Cyclin E1 during hepatocarcinogenesis limits stem cell traits and hepatic myeloid cell homing and attenuates cancer progression

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Background and aims: Hepatocellular carcinoma (HCC) is one of the most severe tumor diseases with increasing incidence and limited treatment options. HCC initiation and progression are associated with persistent proliferation of hepatocytes and non-parenchymal cells. Proliferation is related to cell cycle activity, which is basically regulated by cyclins and Cyclin-dependent kinases (Cdks). E-Type cyclins (Cyclin E1, E2) and their canonical binding partner Cdk2 are key mediators of early cellular DNA replication. Own work demonstrated an essential role of Cyclin E1 and Cdk2 specifically for initiation of HCCs in a murine prevention model (Sonntag *et al*., PNAS 2018 Sep 11;115 (37):9282-9287). In the present study, we investigated the therapeutic benefit of Cyclin E1 or Cdk2 gene targeting after onset of hepatocarcinogenesis (intervention model).
Method: In this study, conditional Cyclin E1 (CcnE1$^{f/f}$) or Cdk2 (Cdk2$^{f/f}$) mice in a C57B6/J background with inducible Cre-recombinase under control of the Mx-gene promoter were used. For HCC induction, 14 days old male mice were intraperitoneally (*i.p.*) injected with diethylnitrosamine (DEN). Cre-negative littermates were used as controls. After 22 weeks (stage of early HCC), interventional inactivation of Cyclin E1 or Cdk2 in hepatocytes and the hematopoietic cell compartment was performed by three *i.p.* injections of poly-I:poly-C. Two weeks and 16 weeks after intervention, mice were analyzed for tumor burden, proliferation, stemness, DNA repair and the microenvironment composition to determine immediate and long term treatment effects.
Results: At the age of 40 weeks, interventional inactivation of Cyclin E1 resulted in a significant reduction of tumor numbers and size compared to DEN-treated control mice. This finding was associated with a decreased overall hepatic proliferation and intratumoral down-regulation of cell cycle activators, tumor markers, stem cell traits and vascularization. Already two weeks after intervention, Cyclin E1 deletion significantly reduced the expression of pro-proliferative genes. Importantly, Cyclin E1-independent growth in remnant tumors was associated with sustained expression of DNA repair genes. Moreover, Cyclin E1 inactivation also changed the composition of the myeloid HCC microenvironment (*e.g.* myeloid-derived suppressor cells) pointing to a new role of Cyclin E1 for immune progenitor cell homing during liver cancer development. In sharp contrast, interventional inactivation of Cdk2 after onset of hepatocarcinogenesis did not reveal any beneficial effect on tumor burden.
Conclusion: Cdk2 is essential for HCC initiation, but surprisingly fully dispensable for HCC progression. However, interventional inactivation of Cyclin E1 during early HCC progression attenuates disease development. Hence, Cyclin E1 presents a promising therapeutic target for treatment of HCC patients.

P03-11YI AXL positive myeloid cells are expanded within tumours and in the peripheral circulation in patients with hepatocellular carcinoma

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Background and aims: Axl, a member of the TAM family of receptor tyrosine kinases, is known to play key roles both in the regulation of innate immune responses and in mechanisms of resistance to chemotherapy. As such, blockade of its signaling is an attractive therapeutic target in hepatocellular carcinoma (HCC). Recent clinical trials of cabozantinib, an inhibitor of a group of kinases including Axl, have shown promising results. We aimed to identify if regulatory Axl positive immune cells are a significant component of the Axl signature in HCC.
Method: Patients with HCC were recruited at two liver centres. Fresh liver tissue was obtained from patients undergoing resection or transplantation and mononuclear cells were isolated from both viable tumour tissue and background liver by mechanical dissociation. Peripheral blood samples were taken and mononuclear cells were isolated by density gradient centrifugation (Ficoll-Paque); serum was also collected and stored. Cells obtained from both tissue and peripheral blood were immune phenotyped using multi-colour flow cytometry. Formalin fixed paraffin embedded tissue samples from tumour and paired non-tumour background liver were sectioned and immunohistochemical staining performed using antibodies to Axl and CD68; analysis was undertaken using Nuance imaging software. ELISA was used to detect soluble Gas-6 in sera.
Results: Axl positive macrophages are significantly expanded within the HCC tumour infiltrating immune cell population when compared to paired background liver samples (19.6% vs 6.2%; n = 7, p = 0.0073). This was in keeping with immunohistochemical analysis, demonstrating an elevated number of dual positive (CD68 and Axl) cells within the tumour nests when compared to background liver (mean 36 vs. 14 cells per high-powered field, n = 3, p = 0.0635). In the periphery there is a significantly higher proportion (19.4%, n = 14) of circulating Axl positive monocytes in HCC on a background of cirrhosis when compared with patients with cirrhosis without HCC (4.5%, n = 6). Circulating levels of Gas-6 are significantly higher in HCC on a background of cirrhosis (n = 33) vs patients with cirrhosis but without HCC (n = 11) (median 7150pg/ml vs 714.8pg/ml; HCC vs cirrhosis without HCC, p <0.0001).
Conclusion: There is a significant expansion of both intra-tumoural macrophages and circulating monocytes expressing Axl tyrosine kinase in patients with HCC. In addition, there is a pronounced elevation in circulating levels of the principal ligand for Axl signaling, Gas-6. Axl signaling via Gas-6 has been shown to induce an immune regulatory phenotype in myeloid cells. As such, these cells may represent a significant immune suppressive population and therapeutic target in HCC.

P03-12YI Hepatocellular carcinoma systemic therapy: predictive factors of sorafenib benefit

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Background and aims: Sorafenib is a multikinase inhibitor acting on the vascular endothelial growth factor receptor, among others. It is the first line therapy for systemic treatment of unresectable hepatocellular carcinoma (HCC). Multiple prognosis factors associated with sorafenib therapy have been described. Our aim was to analyse HCC patients treated with sorafenib for overall survival and check for predictive factors of sorafenib benefit.
Method: A retrospective observational study was performed. Data from all patients treated in a Liver Unit with sorafenib for HCC from 2008 up to 2016 was collected and analysed. Statistical analysis was performed using SPSS Statistics 23®.
Results: Sixty-two patients were treated with sorafenib for HCC; 83.6% were male; mean age was 63.6 (± 9.9); 44.6% Child-Pugh A cirrhosis and 80.6% were BCLC C. Macrovascular invasion was present in 26.2% and 29.5% had other extrahepatic metastasis. Mean time to progression (TTP), evaluated by mRECIST, was 5.6 months (± 4.2) and mean overall survival (OS) was 12.8 months (± 2.0).
Using bivariate analysis lower alpha-fetoprotein, smaller lesion size, absence of metastasis and use of locoregional therapy (LRT) were statistically associated with survival at 6 months. To test this association, binary logistic regression models were used and adjusted for age, diabetes, Child-Pugh, MELD-Na and BCLC scores. Smaller lesion size and LRT were independently associated with survival at 6 months, the latter with a RR of 10.8 (p = 0.048; 95% CI 1.024-114.5). The absence of macrovascular invasion was associated with OS (p = 0.007), but it was not statistically associated with survival at 6 months.
Lower alpha-fetoprotein, smaller lesion size, absence of metastasis and use of LRT was associated with TTP as well. This association was tested with binary logistic regression models adjusted for the previously described confounders. Smaller lesion size and LRT were also independently associated with TTP greater than 3 months, the latter with a RR of 5.1 (p = 0.042; 95% CI 1.06-24.82).
Neutrophil-to-lymphocyte ratio, albumin/bilirubin ratio, number of lesions, sorafenib dose and other etiology of cirrhosis were not statistically associated with OS or TTP in this sample.
Conclusion: The mean OS in this sample was better than the described in SHARP study and similar to subsequent real-life studies. In this sample, lesion size and LRT were independently associated with survival at 6 months and TTP greater than 3 months.

P03-13 CEUS pattern of hepatocellular carcinoma: prognostic implication

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Background and aims: American College of Radiology (ACR) has released the Liver Imaging Report And Data System (LI-RADS) scheme, which categorizes nodules in patients at risk for hepatocellular carcinoma (HCC) according to the degree of risk of nodules to be HCC as LR-3, LR-4, LR-5 (definitely HCC) and LR-M (probable malignancy not specific for HCC). EASL guidelines recommend biopsy for nodules not meeting the LR-5 class whereas LI-RADS policy, adopted by AASLD, largely include only imaging follow-up.
Aim of this study was to test whether HCC showing the CEUS pattern of indeterminate nodules (LR-3 and LR-4) are associated with better prognosis in terms of *Overall Survival* (OS) and *Recurrence-Free Survival* (RFS) than LR-5 (which could support LI-RADS/AASLD policy) or not (which would mandate EASL policy).
Method: Among 472 consecutive cirrhotic patients with liver nodules referred to our Centre (January 2005-December 2016), we retrospectively enrolled 98 patients with first diagnosis of single HCC according to 2012 AASLD guidelines (CT/MRI if typical or histology if CT/MRI were inconclusive) for whom a complete CEUS pattern, categorized according to the CEUS LI-RADS policy, was available.
Results: Median size of HCC lesions was 2.5 cm (range 1-7.2 cm). According to CEUS LI-RADS classification, 8 (8%) patients were in LR-3, 31 (32%) in LR-4, 54 (55%) in LR-5 and 5 (5%) in LR-M. Patient and nodule characteristics were not statistically different between LI-RADS classes. At univariate analysis CEUS LI-RADS class was not found to be a predictor of survival but statistically related to RFS ($p = 0.029$), since LR-M had shorter RFS than other classes. Also the LR-4 had shorter RFS than LR-5.
Conclusion: HCC showing the CEUS LI-RADS classes LR-3 and LR-4 have no better clinical outcome than typical HCC, therefore conclusive diagnostic investigation of indeterminate nodules up to liver biopsy is promptly requested to avoid leaving aggressive HCC not timely treated.

P03-14YI Efficacy and safety of thermal ablation in patients with hepatocellular carcinoma (HCC) and high comorbidity (hc)

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Background and aims: Radiofrequency (RF) is the ablative method of choice in HCC BCLC-0/A not candidates for resection or transplantation. Microwave ablation (MW) is potentially more effective by avoiding the heat sink effect and achieving greater ablation volume. There is little evidence comparing RF vs MW (specially in Caucasian patients) and also about the prognosis in patients (pat.) with high comorbidity (hc). Describe the efficacy and safety of thermal ablation in pat. with hc (Charlson index>3) with HCC.

Method: This is a retrospective observational unicentric study on pat. with HCC and high comorbidity who received RF/MW as initial treatment, decided on a multidisciplinary committee (MC). Visits were made at baseline, one more post-RF/MW and every 4 months if evidence of a complete response (mRECIST criteria), deciding the follow-up schedule in MC. Baseline variables (comorbidity, liver function, tumour burden), type of treatment, adverse effects, and clinical-analytical-radiological changes of each visit were recorded. RF was performed with cool-tip RF Ablation System (Medtronic) and MW with Solero MTA System (AngioDynamics) under deep sedoanalgesia.

Results: From Jan 2014 to July 2018, 163 pat. were treated and 86 met the inclusion criteria (44 RF/42 MW): 70 males, median age 69, range 50-85 years; 74 cirrhosis (66 Child-A, 8 Child-B7); Charlson index: median 8, range 4-14. Etiology: 39 alcoholic, 34 HCV, 6 NAFLD. Staging: 19 BCLC-0/67 BCLC-A; 70 uninodular, 50 subcapsular, median tumour diameter 25mm (P25- P75 19-33). Complete response to the first ablation was observed in a 65.9%, being the most frequent recurrence adjacent to the ablation margin (73, 04%). There were 14% complications (decompensation of cirrhosis 2.3%), 1 death after colon perforation. Baseline characteristics, tumour burden and evolution were similar in RF vs MW, except basal platelets (RF: $128x10^3$vs MW: $131x10^3$, p = 0.006) and nº nodules [RF: 1 (n = 29), 2 (n = 11), 3 (n = 2) vs MW: 1 (n = 41), 2 (n = 3), 3 (n = 0), p = 0.012]. No differences were found in the complete response rate (p = 0.203) or in complications (p = 0.412). Median follow-up was 19 months, 12 transplanted, 16 deceased (10 due to comorbidity). The median overall survival, OS (n = 86) was 40 months, 95% CI 37.4-42.6, without differences between RF/MW (p = 0.234). The baseline factors associated with OS in the univariate analysis were age (p = 0.024), platelets (p <0.01), serum creatinine (p <0.01), Charlson index (p = 0.008), BCLC (p = 0.096) and AFP (p <0.01); and in the multivariate BCLC (p = 0.022, HR 4.090, 95% CI 1.230-13.597), the Charlson index (p = 0.014, HR 0.104, 95% CI 0.017-0.635) and the AFP value (p = 0.012; HR 1.005; 95% CI 1.001-1.009).

Conclusion: Thermal ablative treatments are safe and effective in patients with high Charlson index. In our series, there were no differences in the complete radiological response rate or in the adverse events between RF and MW.

P03-15 Early experience with lenvatinib for the treatment of advanced hepatocellular carcinoma in real world practice in Japan

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Background and aims: Lenvatinib (LEN) therapy for unresectable hepatocellular carcinoma (u-HCC) has been approved since Mar 2018 in Japan. In the phase 3 trial (REFLECT trial), patients who had experienced any systemic therapy were excluded. However, in real world practice lenvatinib therapy has been performed as a third-line treatment. We investigated the efficacy and safety in u-HCC patients treated with lenvatinib in real world practice in Japan.
Method: A total of 34 u-HCC patients received lenvatinib from Apr 2018 at our institution were included. Tumour assessments in accordance with modified RECIST were done using dynamic CT or MRI within 4-8 weeks and every 6-8 weeks thereafter. Adverse events (AEs) were graded according to the CTCAE ver4.0.
Results: Median age was 75 (52-88) years, 31 (91.2%) patients were male, and median body weight (BW) was 60 (30-94) kg. The baseline liver function was Child-Pugh class A in 31 (91.2%) patients. Twenty-one (61.7%) patients were BCLC stage C. Eighteen (53.0%) patients received LEN as a first-line agent. Six (17.6%) patients received LEN as a second-line therapy and 3 of the 6 patients showed sorafenib-intolerance. Two of the 6 patients experienced checkpoint inhibitors in a clinical trial and the remaining 1 patient was treated with a cytotoxic agent before LEN. As a third-line agent, 10 (29.4%) patients received LEN and 9 of the10 patients experienced sorafenib-regorafenib sequential therapy and 1 patient received brivanib and sorafenib before LEN. Median observation time was 4.0 months and 6 patients died from HCC progression. The imaging findings of 28 (85.3%) patients were evaluated. Based on mRECIST, CR was shown in 5 (17.2%), PR in 6 (20.7%), SD in 12 (41.4%), and PD in 5 (17.2%). ORR and DCR of tyrosine kinase inhibitor (TKI) naïve patients (n = 15) were 46.7% and 73.3%, while those of TKI experienced (n = 13) were 30.8% and 92.3%. Fourteen patients started 12mg/day and dose reduction was necessary in 92.9% of the patients. The common any-grade AEs were hypertension (61.8%), diarrhoea (20.6%), decreased appetite (55.9.%), fatigue (58.8%) and proteinuria (35.3%). Hand-food skin reaction (HFSR) was observed in 32.4% of patients. In patients with BCLC stage B (n = 13), the incidence of fatigue was higher in elderly patient (>80 years, n = 7) than the other patients (85.7% vs 50.0%).
Conclusion: The efficacy of LEN in real world practice was similar to the phase 3 trial. The excellent antitumor effect has been reported in the patients who experienced TKI therapy before LEN, although the number of the patients was still very small. Appetite loss and fatigue must be carefully monitored and well managed during LEN therapy, especially in elderly patients.

P03-16YI Combination of ubiquitin carboxy-terminal hydrolase L1 inhibition and Sorafenib treatment in experimental hepatocellular carcinoma dampens tumor aggressiveness and reduces in vitro functional liver cancer stem cell characteristics

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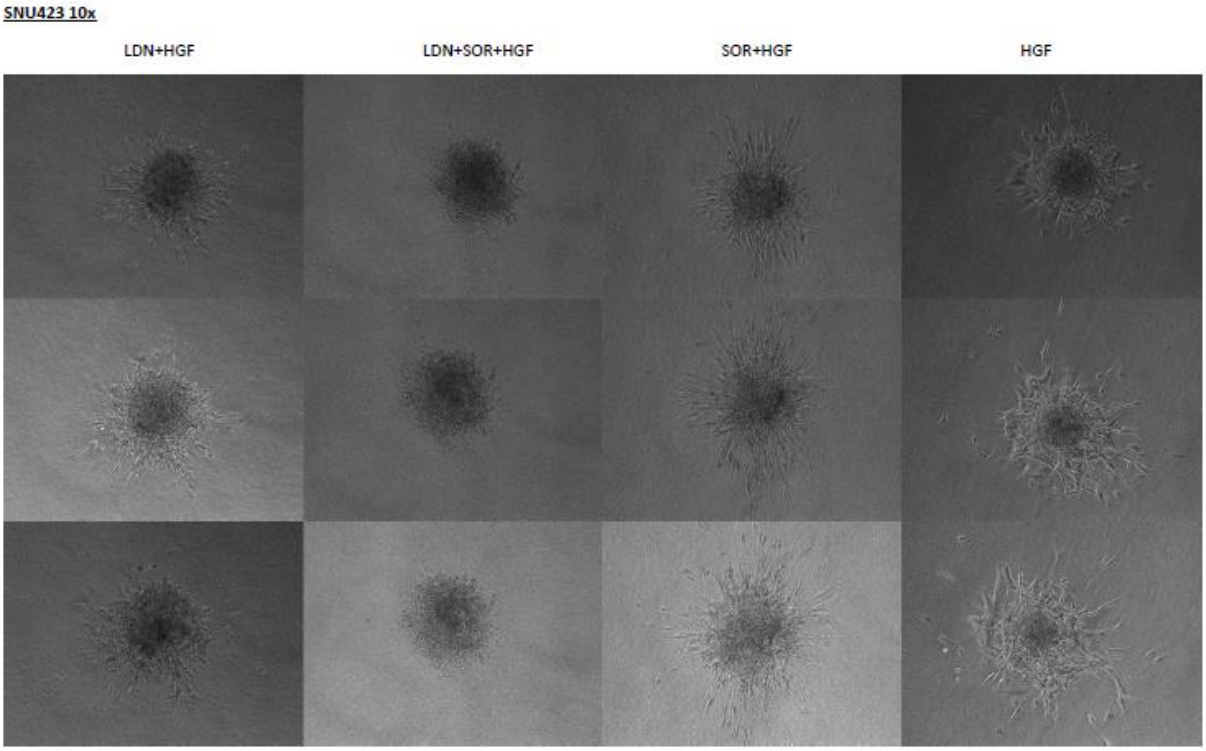
Background and aims: Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related mortality worldwide. In the majority of patients with advanced HCC, therapy with the golden standard sorafenib (SFN) has limited efficacy with frequent adverse events and often results into aggressive relapse. It has been put forward that liver cancer stem cells (LCSC) play a pivotal role in therapy resistance, invasion, metastasis, and recurrence of HCC. Consequently, treatment strategies combining SFN with targeted compounds to potentially diminish LCSC properties are an unmet medical need. Ubiquitin carboxy-terminal hydrolase L1 (UCHL1) is a key regulator of protein homeostasis and has been reported to be deregulated in HCC. However, its role as a tumor promoter or suppressor remains controversial. In this study, we investigated the potential of the UCHL1 inhibitor LDN57444 (LDN) compared to and with SFN treatment on functional stemness features.

Method: Orthotopic multifocal HCC in mice was established by N-nitrosodiethylamine (DEN) injections for 25 weeks. Ectopic human HCC xenografts were obtained by subcutaneous injection of 5x10^6 human Hep3B cells in nude mice. Animals were treated with 10 mg/kg SFN (daily), 0.4 mg/kg LDN (biweekly), combined therapy or vehicle for 5 or 3 weeks (DEN or xenograft model, respectively). Expression of HCC markers was evaluated in ectopic and orthotopic tumors, and corresponding non-neoplastic liver tissue by RT-qPCR. Tumor cell proliferation and viability were assessed by histological analyses (Ki-67 and HandE) of the ectopic tumors and validated *in vitro* by MTT and LDH assays. Assessment of functional LCSC characteristics was performed *in vitro* by colony formation and 3D spheroid invasion assays.

Results: Although no significant differences in tumor burden (DEN model) or volume (xenograft) were observed, similar survival benefits and reduction of intra-tumoral HCC marker expression was observed in ectopic and orthotopic HCC mice treated with SFN or the combination therapy. Xenograft tumors were less aggressive upon combination therapy, with reduced numbers of proliferative Ki-67 positive cells and enhanced necrotic tumor centers. This was confirmed *in vitro* with significant loss of viability and cell integrity in Hep3B cells treated with both SFN and LDN compared to cells treated with SNF or LDN alone. Interestingly, combo-treated cells displayed less functional LCSC features shown by reversed SFN-induced colony formation and enhanced attenuation of spheroid invasiveness in Hep3B and SNU423 cells.

Conclusion: Combination of UCHL1 inhibition and SFN treatment in HCC mice dampens tumor aggressiveness. Inhibiting UCHL1 in SFN-treated HCC cells reduces functional LCSC characteristics. Further *in vivo* studies using a metastatic HCC xenograft model will have to reveal if this strategy is able to subdue HCC metastasis.

Figure:



P03-17YI Color Doppler and contrast-enhanced ultrasonography to manage hemorragic complications occurring after percutaneous thermal ablation of hepatocellular carcinoma.

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Background and aims: To evaluate the usefulness of color Doppler ultrasonography (CD-US) and contrast-enhanced US (CE-US) in the management of hemorrhagic complications after percutaneous thermal ablation (PTA) of hepatocellular carcinoma (HCC).
Method: From January 2010 to January 2018, 685 cirrhotic patients (454 M; Child-Pugh score ≤B7) aged 69 ± 7 years (range, 56-81 years), with a single, naïve, HCC nodule ≤3.0 cm in diameter underwent US-guided PTA. All patients had safe coagulation parameters. The HCC nodules were treated in 920 PTA sessions with an average of 1.3 (range, 1-4) active needle (RF electrodes/MW antennas) insertions per nodule. CD-US, with/without spectral analysis (SA), and CE-US were performed on the needle path/s soon after each session.
Results: Arterial bleeding was observed in 7/920 (0.8%) PTA sessions: six due to tearing of a hepatic artery brunch and one of the inferior epigastric artery. CD-US showed evaluable color signals along the needle path in 87/920 (9.4%) cases: 80/87 (91.9%) self-limiting bleeding episodes showed a venous pattern at SA and 7/87 (8.1%) an arterial pattern. CE-US, performed in 906/920 (98.4%) sessions (14 patients had contraindications to sulfur fluoride injection), showed an enhanced needle path in the early arterial phase in seven cases. The stop of arterial bleeding was achieved by hepatic artery embolization in six patients and by US-guided injection of N-butyl-2-cyanoacrylate added of iodinated contrast medium within artery in the remaining one.
Conclusion: CD-US and CE-US allowed early diagnosis and successful treatment of arterial bleeding in all cases, proposing themselves as mandatory examinations soon after each HCC PTA session.

P04-01YI SIRT-3, p-mTOR and HIF-1 in hepatocellular carcinoma:implication in metabolica dysfunctions and prognostic significance

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Biological and clinical role of SIRT-3 and its downstream effectors in Hepatocellular carcinoma: implication in metabolic dysfunctions and prognostic significance.

Background and aims: In the last years, HCC cases deriving from metabolic dysfunctions such as type 2 diabetes mellitus (T2DM), metabolic syndrome and non- alcoholic steatohepatitis are increased.
SIRT-3 is a key player in the regulation of different processes such as metabolism and cancer. However, its role in HCC metabolism as well as development and progression remains unclear yet.
This study aims to better clarify the biological and clinical function of SIRT-3, p-mTOR and HIF-1α and its downstream targets in early and advanced stage HCC patients, in relation to the presence of metabolic alterations and clinical outcome.
Method: A total of 70 HCC patients of which 48 cases at early stage and 22 cases at advanced stage was enrolled. The expression of SIRT-3, HIF-1α and p-mTOR was evaluated by immunohistochemistry using the Ventana Benchmark XT staining system and summarized using descriptive statistics (absolute and relative frequency, means, standard deviation, median and quartiles).
Results: Relatively to aetiology (viral *vs* metabolic syndrome *vs* other), the presence of T2DM and treatment with metformin, SIRT-3 expression resulted higher in patients with diabetes (median value of 60%) than non diabetic patients (median value of 30%) ($p = 0.011$) and in patients treated with metformin than in those taking insulin (70% *vs*. 30%, respectively) ($p = 0.030$).
Interestingly, p-mTOR resulted more expressed in patients with metabolic syndrome (median value of 0% with a range of positivity in the neoplastic population varying from 0% to 100%) compared to those with different aetiology ($p = 0.036$), in diabetic patients treated with metformin than those taking insulin (median value of 0% with a range from 0% to 100% *vs* 0% with a range from 0% to 40%) ($p = 0.021$).
Relatively to clinical outcome, in the setting of early stage HCC patients, the trend was in favor of patients with high expression of SIRT-3 and HIF-1α. Instead, in the setting of advanced stage patients, we observed that p-mTOR correlated with unfavorable clinical outcome.
Conclusion: Our study highlights the key role of SIRT-3 and p-mTOR in HCC metabolism. Relatively to their involvement in the development and progression of tumor, we suggest SIRT-3 and HIF-1α as predictor of prognosis in early stage HCC patients, whereas p-mTOR as promising therapeutic target for the treatment of advanced stage HCC.

P04-02YI Hepatic epithelioid hemangioendothelioma: an international multicenter study

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Background and aims: Hepatic epithelioid hemangioendothelioma (HEHE) is a vascular neoplasm, more frequent in women than in man and usually arises in a non-cirrhotic liver. This is a very rare tumor (0.1-1/100, 000 person) and no prospective study on this population is available until today. The aim of this study is to describe the HEHE patients profile and the treatment of patients with this orphan condition.

Method: We performed an international (Sao Paulo, Salvador de Bahia and Barcelona-Clínic), retrospective and multicentric study registering the baseline clinical, biochemical radiological and pathological features and all the evolutionary events that were available in the patient file. The images were centralized and pathologic samples were revised by three pathologists (MS, VA and CM) with more than 10 years of experience in liver cancer.

Results: baseline clinical and laboratory data were available in 25 patients. One patient was diagnosed of a hepatic angiosarcoma at the moment of liver transplantation (LT) and finally excluded from this analysis. Median age was 38.7 years, the majority of patients were females (64%), without underlying chronic liver disease (76%). In 17 patients the diagnosis was incidental while 7 patients were symptomatic. Of the 24 included patients, 66.7% had multinodular disease and 50% presented extrahepatic disease at the diagnosis, all of them presenting at least lung involvement. Interestingly, 71% of the patients received a specific treatment such as chemotherapy (n:6), resection (n:7) or LT (n:4). The pathology revision was possible in 20 patients and the central radiologic evaluation only in 13 patients. The most frequent radiologic pattern, was a progressive central contrast uptake in 6 patients, followed by stable peripheral enhancement without changes through the phases and persistent minimal uptake through all phases in 4 and 3 patients in each pattern, respectively. Median follow-up in the whole cohort was 80.2 months [IQR; 51.7 to 154.3], survival rate at 5- and 10-years were 91.5% and 51.9%, respectively. Eleven patients of the whole cohort developed progression and 7 died.

Conclusion: This multicentric study reflects the need of a better characterization and understanding of the natural history of this hepatic neoplasm. Active research through larger international consortia should define the molecular pathways involved in its development and allow an optimal treatment approach.

P04-03YI Validation of modified ALBI-T score as a prognostic model to evaluate Egyptian patients with hepatocellular carcinoma

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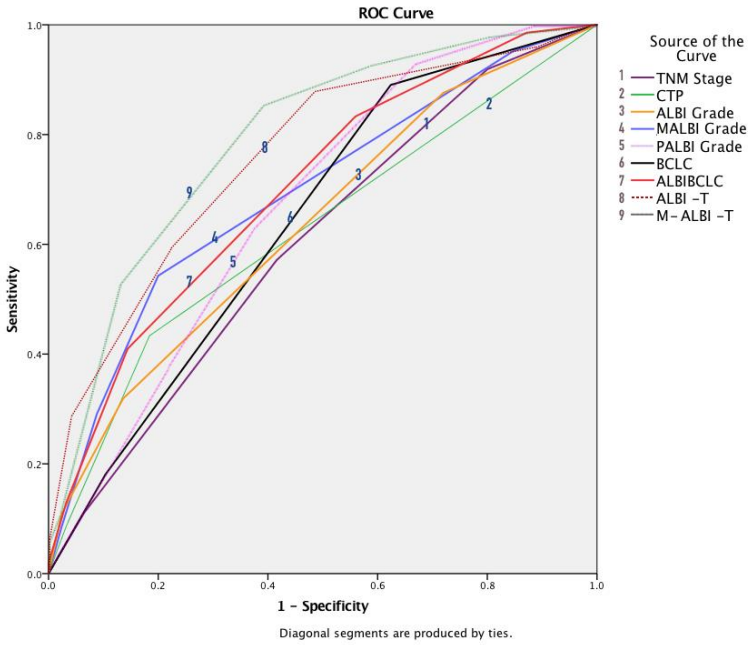
Background and aims: An ideal staging system for hepatocellular carcinoma (HCC) should rely on the hepatic reserve function and tumor burden. With improving diagnostic and treatment strategies for HCC, in addition to recent treatment of viral hepatitis, finding a suitable assessment tool for hepatic reserve has become mandatory. This study aimed to validate the recently proposed modified albumin-bilirubin-TNM (mALBI-T) grade as a prognostic model for patients with HCC in Egypt.
Methods: A data set of a cohort of 1910 patients diagnosed with HCC and fulfilled the inclusion criteria was accrued from the HCC clinic at National Liver Institute, Menoufia University. CTP, BCLC, ALBI, PALPI, ALBI-based BCLC, ALBI-T and modified ALBI-T grades were estimated. Patients were followed up from the time of diagnosis to the date of death or date of data collection if they remained alive. The overall survival and the received treatment were determined. Survival data were analyzed using Kaplan Meir survival curves and log rank test then area under ROC was determined.
Results: For 1910 patients, the mean age was 57 years, 1575 were males. At presentation, 50.6% were CTP A, 36.1% were CTP B and 13.4 % were CTP C. Most of patients were ALBI grade 2 (63.2%), 17.8 % were ALBI grade 2A while 45.5% were ALBI grade 2B. ALBI grade 1 and 3 were 12% and 24.7% respectively. The overall median survival was 13 months; the median survival was better in patients with ALBI grade 1 than ALBI 2 and 3 (28.6 vs. 14 and 5.8 months respectively, P <0.001). Moreover, the median survival for ALBI grade 2A patients was better than ALBI 2B (18.6 vs. 13 months respectively, P <0.001). ALBI-T grades 0 and1 patients had better median survival than those of ALBI-T grades 2, 3, 4 and 5 (42, 28.9, 17, 8, 5 and 3 months respectively (p <0.001). The modified ALBI-T showed better stratification and significant improvement in the median survival of modified ALBI-T grades 2, 3, 4, 5 and 6 to be 20, 14, 8, 5 and 3 months respectively.
Conclusion: ALBI-T grade is a superior total prognostic tool that selects patients with HCC who have better liver reservoir and tumor stage; this ability increases by sub-classification of ALBI grade 2 . Modified ALBI-T may be the ideal overall prognostic model in patients with HCC**.**
Figure:



P04-04YI Oncological long term benefit in case of anatomic versus parenchyma-sparing resection for hepatocarcinoma: does the extension matter? A meta-analysis of high quality propensity matched and randomized studies.

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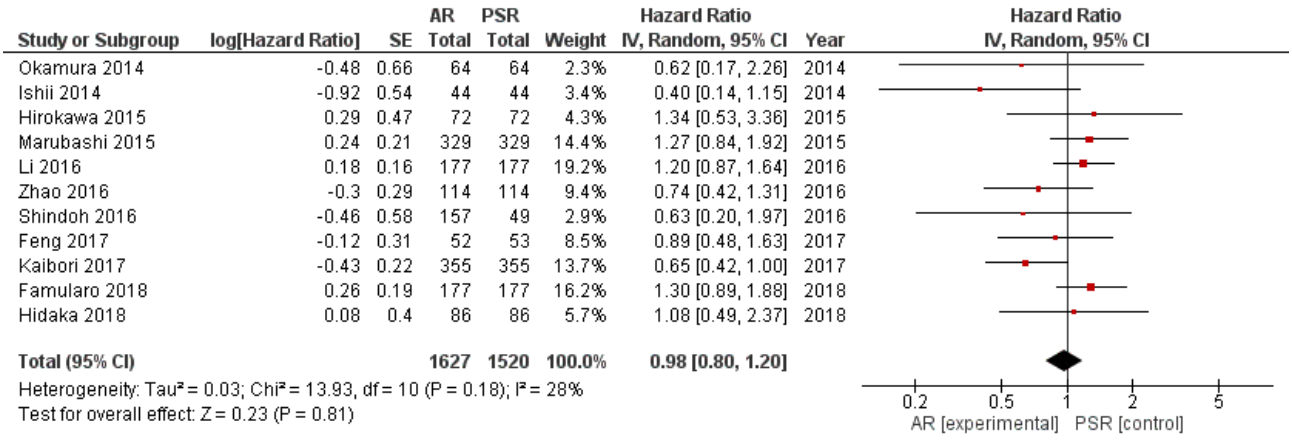
Background and aims: The benefit of Anatomic (AR) versus Parenchyma-sparing resection (PSR) in hepatocarcinoma (HCC) is still debated. It has been also highlighted a significant clinical heterogeneity among studies, since the great majority of data is derived from retrospective case series with significant differences between groups. The aim of the study was to meta-analyze the available results of AR vs PSR in terms of overall-survival (OS) and disease-free-survival (DFS) only in high quality, well matched studies.
Method: A systematic review and meta-analysis was conducted using Medline, clinicaltrial.gov and Google Scholar through April 30th 2018. To overcome selection bias in retrospective studies and reducing intra- and inter-study heterogeneity, only propensity-matched studies and randomized clinical trials comparing AR vs PSR were deemed eligible. Inter-study heterogeneity was assessed by Higgins I^2 statistic for the rate of Child A, female patients, presence of hepatitis B and C, number and size of nodules between each group. Log-transformed HRs were pooled in a generic inverse variance meta-analysis. Review Manager (RevMan) software version 5.3 was employed for the analyses.
Results: Eleven propensity-matched and 1 RCT were enrolled, with a total of 3445 patients (AR = 1776 and PSR = 1669). Rate of Child A, females, presence of hepatitis B and C, number of nodules and size were comparable intra and inter studies (I^2<50%, p >0.5). No significant difference was evident in terms of OS between AR and PSR at 1 year (RR 1.07; 95%CI: 0.83-1.37; p:0.62; I^2 = 8%), at 3 years (RR 0.98; 95% CI: 0.80-1.20; p:0.81; I^2 = 28%) and at 5 years (RR 0.89; 95%CI: 0.74-1.07; p:0.21; I^2 = 66%). Overall risk of mortality was comparable between the two procedures (HR 0.98; 95%CI: 0.80-1.20; p:0.81, I^2 = 28%). Furthermore, overall risk of recurrence was not significant (HR 0.84; 95%CI: 0.70-1.00; p:0.051, I^2 = 61%). No differences on DFS were evident at 1 year (RR 0.87; 95%CI: 0.76-1.00; p:0.06; I^2 = 31%), 3 years (RR 0.95; 95%CI: 0.44-1.08; p: 0.43; I^3 = 75%) and at 5 years (RR 0.95; 95%CI: 0.89-1.01; p:0.08; I^2 = 36%).
Conclusion: When liver function and tumor burden are comparable, and the intra and inter-study heterogeneity is low, AR and PSR reach identical long terms outcomes. However, the anatomical removal of the portal bearing seems to show a non-significant but present tendency to improve local control of the disease in the early period after surgery.
Figure:



Study or Subgroup	log[Hazard Ratio]	SE	AR Total	PSR Total	Weight	Hazard Ratio IV, Random, 95% CI	Year
Okamura 2014	-0.48	0.66	64	64	2.3%	0.62 [0.17, 2.26]	2014
Ishii 2014	-0.92	0.54	44	44	3.4%	0.40 [0.14, 1.15]	2014
Hirokawa 2015	0.29	0.47	72	72	4.3%	1.34 [0.53, 3.36]	2015
Marubashi 2015	0.24	0.21	329	329	14.4%	1.27 [0.84, 1.92]	2015
Li 2016	0.18	0.16	177	177	19.2%	1.20 [0.87, 1.64]	2016
Zhao 2016	-0.3	0.29	114	114	9.4%	0.74 [0.42, 1.31]	2016
Shindoh 2016	-0.46	0.58	157	49	2.9%	0.63 [0.20, 1.97]	2016
Feng 2017	-0.12	0.31	52	53	8.5%	0.89 [0.48, 1.63]	2017
Kaibori 2017	-0.43	0.22	355	355	13.7%	0.65 [0.42, 1.00]	2017
Famularo 2018	0.26	0.19	177	177	16.2%	1.30 [0.89, 1.88]	2018
Hidaka 2018	0.08	0.4	86	86	5.7%	1.08 [0.49, 2.37]	2018
Total (95% CI)			**1627**	**1520**	**100.0%**	**0.98 [0.80, 1.20]**	

Heterogeneity: Tau² = 0.03; Chi² = 13.93, df = 10 (P = 0.18); I² = 28%
Test for overall effect: Z = 0.23 (P = 0.81)

P04-05YI Analysis of the HSD17B13:TA allelic variant as a protective factor towards hepatocellular carcinoma in patients with and without chronic hepatitis C

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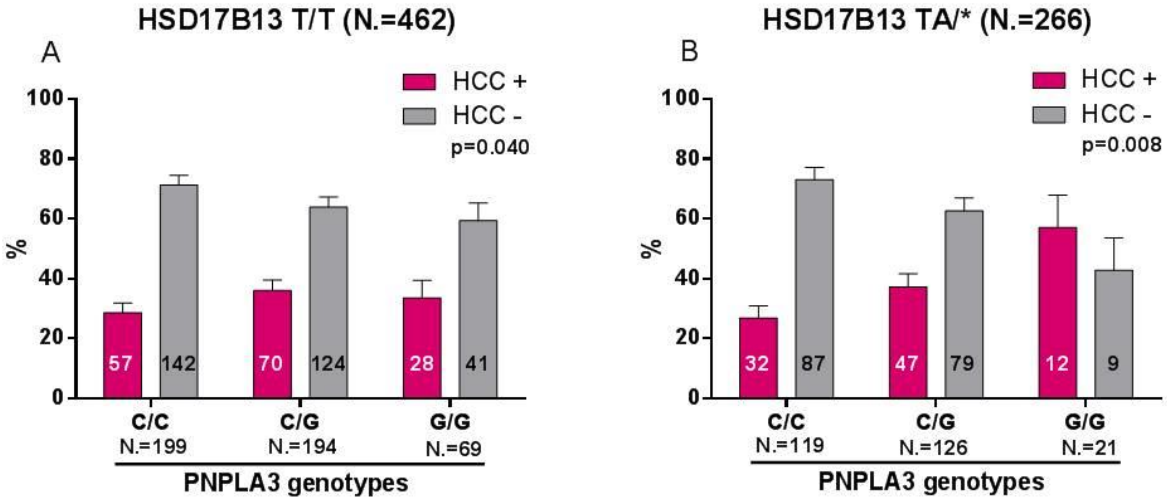
Background and aims: The PNPLA3 single nucleotide polymorphism rs738409 (C>G) is a major genetic factor for steatosis, fibrosis progression and hepatocellular carcinoma (HCC). In fact, carriers of the G allele with chronic liver disease are more likely to progress to cirrhosis and HCC, especially when affected by alcoholic or non-alcoholic fatty liver disease (NAFLD). However, these same patients have been shown to be relatively protected against developing cirrhosis by carriage of the HSD17B13:TA variant (rs72613567) (Abul-Husn NS et al. N Engl J Med. 2018;378:1096-106). Since cirrhosis is by far the strongest risk factor for HCC, it is conceivable that such protection might extend to HCC. We aimed to verify this hypothesis.
Method: The study population included N. = 728 patients, among whom N. = 246 had a diagnosis of HCC (Group 1; 135 with hepatitis C virus infection, HCV, 55%); N. = 180 HCV infected patients (Group 2) and 302 NAFLD (Group 3) patients had chronic liver disease with and without advanced fibrosis/cirrhosis, not complicated by HCC. Restriction fragment length polymorphism analysis was performed to determine the allelic variants frequency of PNPLA3 and HSD17B13.
Results: The PNPLA3:G frequencies were 0.40, 0.31 and 0.32 for Group 1, Group 2 and Group 3, respectively (p = 0.004). The HSD17B13:TA frequencies were 0.21, 0.28 and 0.16 for Group 1, Group 2 and Group 3, respectively (p <0.001). The figure shows the distribution of PNPLA3 genotype frequencies, according to the presence or absence of HCC and HSD17B13 status. By considering only the subgroup of patients negative for HCV (N. = 416/728, 57%), similar results were observed: based on PNPLA3 genotype, patients with HCC were 22/118 (19%) vs. 10/55 (18%) (C/C), 35/131 (27%) vs. 21/55 (38%) (C/G), and 20/45 (44%) vs. 6/12 (50%) (G/G), among HSD17B13 wild-type (p = 0.002) vs. HSD17B13 variant carriers (p = 0.006), respectively.
Conclusion: The HSD17B13:TA allelic variant does not appear to decrease appreciably the risk of developing HCC conferred by carriage of the PNPLA3:G allele, neither among HCV positive nor among HCV-negative patients.
Figure:



P04-06 Galectin 3 as a risk factor for recurrence of Hepatocellular Carcinoma after liver transplantation

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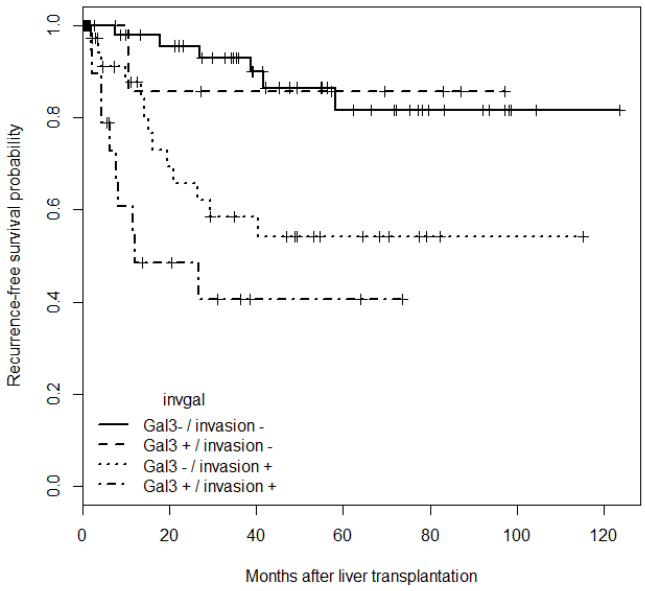
Background and aims: In current clinical practice, hepatocellular carcinoma recurrence occurs in 10% to 20% of patients submitted to liver transplantation. Post-transplant prognosis is based on traditional histopathological data; however, such an approach is limited since it does not consider tumour behaviour. Therefore, it is necessary to identify potential biomarkers that may refine prognosis, improve post-transplant care, and enable early detection of tumour recurrence. Here, we investigated galectin 3 expression in tumour cells from patients with hepatocellular carcinoma submitted to liver transplantation in a referral centre in Northeast Brazil and evaluated its association with histological findings, tumour recurrence, and recurrence free survival.

Method: An ambispective cohort study included 142 patients with hepatocellular carcinoma submitted to liver transplantation and post-transplant follow-up in a Brazilian referral centre. Galectin3 expression was evaluated in tumour nodules from the explanted liver using immunohistochemistry.

Results: After a median follow-up period of 33 months, tumour recurrence occurred in 31 cases (7.92/100 person-years). Galectin3 expression in tumour cells was detected in 36 cases (24.3%) and was independently associated with vascular invasion (OR, 3.13; 95% CI, 1.18-8.29) and previous hepatitis B infection (OR, 3.32; 95% CI, 1.04-10.58). Galectin3 expression, when combined with vascular invasion, was independently associated with a higher post-transplant risk of recurrence (HR, 8.36; 95% IC, 2.99-23.29) and shorter recurrence free survival (*p* <0.001).

Conclusion: Our findings provide new insights into the synergistic effects of Galectin3 and vascular invasion on increased risk of hepatocellular carcinoma recurrence after liver transplantation.

Figure: Kaplan Meier curves of recurrence-free survival in HCC patients submitted to LT according to combined variable Gal3/vascular invasion, *p* log-rank <0.001.



P04-07 Evaluation of LI-RADS v2018 by magnetic resonance in US-detected nodules 2cm in cirrhotics

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Background and aims: Liver Imaging Reporting and Data System (LI-RADS) was developed to classify the observations in six categories from LR-1 (definitively benign) to LR-5 (definitively HCC), stratifying the probabilities of HCC diagnosis to assist the clinicians in the decision making. Since its implementation in 2011, LI-RADS has been updated according the results of several studies evaluating its diagnostic performance. In the last version in 2018, aimed to be implemented by AASLD, nodules between 10-19 mm displaying non-rim arterial phase hyperenhancement and non-peripheral washout were assigned as LR-5. The objective of this study was to evaluate the diagnostic accuracy of LI-RADS v2018 when using magnetic resonance imaging (MRI) for hepatic nodules ≤20mm detected during ultrasound (US) surveillance in cirrhotic patients, with particular interest in those observations categorized as LI-RADS 3.
Method: Between November 2003 and February 2017 we included 262 cirrhotic patients with a newly US detected solitary ≤20 mm hepatic nodule who were prospectively examined by MRI and fine-needle biopsy (reference-standard) and followed-up with MRI every 6 months if initial definitive diagnosis was not achieved. A LI-RADS (LR) category according to v2018 was retrospectively assigned. The diagnostic accuracy for each LR category was described and the main MRI findings associated with HCC diagnosis were analyzed.
Results: Final diagnoses were: 197 HCC (75.2%), 5 intrahepatic cholangiocarcinoma (1.9%), 2 metastasis (0.8%) and 58 benign lesions (22.1%). 0/15 (0%) LR-1, 6/26 (23.1%) LR-2, 51/74 (68.9%) LR-3, 11/12 (91.7%) LR-4, 126/127 (99.2%) LR-5, and 3/8 (37.5%) OM were HCC. LR-5 category displayed a sensitivity of 64.5% (CI95%: 57.4-71.1), very similar to the sensitivity achieved by EASL criteria (62.9% [CI95%: 55.8-69.7]). Considering also LR-4 as diagnostic for HCC, the sensitivity slightly increased to 69.5% (CI95%:62.6-75.9) with minor impact on specificity (96.2%; CI95%:89.3-99.6). Regarding LR-3 observations, 51 out 74 were HCC, 2 were non-HCC malignancies, and twenty-one out 22 LR-3 nodules >15 mm (95.5%) were finally categorized as HCC.
Conclusion: In cirrhotic patients with nodules ≤20 mm detected on US, distinction between LR-4 and LR-5 according to v2018 has minor impact. A relevant proportion of LR-2 and LR-3 lesions corresponded to an HCC and thus, an active diagnostic work-up including biopsy is recommended.

P04-08 Percutaneous Microwave (MW) ablation is better than Radiofrequency (RFA) to obtain complete response in cirrhotic patients with very early and early hepatocellular carcinoma

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Background and aims: Percutaneous thermal ablations are widely employed treatments for hepatocellular carcinoma (HCC). It has been proved that complete necrosis in early HCC and cirrhosis is related to a better survival. We compared the efficacy of RFA and MW to obtain complete response (CR) in patients with HCC.
Method: This retrospective observational study was carried out on a cohort of 227 adult cirrhotic patients with a first diagnosis of early HCC (single node ≤50 mm or ≤3 nodules ≤ 35 mm), consecutively treated with RFA or MW between January 2013 and July 2018 at our Gastro-hepatology Unit. Diagnosis and treatment allocation followed EASL guidelines. The choice of RFA or MW was reliant on operators and local availability. Other treatments and combined therapies were excluded. Complete response (CR) was evaluated with multiphasic contrast-enhanced computed tomography (CT) or Magnetic Resonance Imaging (MRI) at 5-7 weeks after treatment and in the follow-up, relying on mRECIST criteria.
Results: 227 subjects (M/F 163/64, viral/non viral etiology 181/46, median age 63 years [IQR 56-73]) were included. A total of 295 nodules treated with RFA (212, 72%) or MW (83, 28%) was evaluated. However, a homogeneous subgroup of 184 patients with 220 comparable nodules in terms of diameter (15-35 mm) were considered. The two groups were also homogeneous for age, BMI, Child Pugh score, smoke and alcohol habits, complex position, ultrasound visibility and number of nodules per each patient. Overall, a complete response was achieved in 54/58 (93%) HCC after MW and in 126/158 (80%) HCC after RFA (OR 3.43; IC95% 1.14-10.3, p = 0.028). In 15-20 mm nodules, the two procedures were not significantly different (OR 3.41; IC95% 0.40-29.2, p = 0.264), however in 21-35 mm nodules MW was significantly superior in obtaining CR compared to RFA (OR 3.70; IC95% 1.00-13.7, p = 0.05). The rate of CR was inversely proportional to the number of nodules, but without statistical significance. Multivariate Cox-proportional hazard regression showed that MW was an independent predictor of CR in HCC nodules (OR 3.92, IC95% 1.26-12.2, p = 0.018).
Conclusion: in this study MW appeared to be superior to RFA to induce complete response in patients with very early and early HCC with major evidence in bigger nodules (21-35 mm). Further studies are ongoing to confirm this findings and to evaluate the effect on survival.

P04-09YI A multicentric study on real-life impact of nivolumab in patients with hepatocellular carcinoma

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Background and aims: Nivolumab was granted accelerated approval by FDA for hepatocellular carcinoma (HCC) based on the results of the Checkmate 040 trial. We aim to describe the clinical characteristics, safety profile and outcomes of patients with HCC treated with nivolumab outside clinical trials (compassionate use).

Method: This is a retrospective, observational, multicentric study involving 10 healthcare centers with multidisciplinary teams led by hepatologists. Clinical and laboratorial data, previous treatments, adverse events (AE) and overall survival (OS) were recorded.

Results: 110 patients received nivolumab, of whom 74 in clinical trials and 36 outside clinical trials. Nivolumab was the first (1L), second (2L) and third-line (3L) treatment in 4 (11.1%), 18 (50%) and 14 (38.9%) patients, respectively. Sorafenib was the 1L in 100% of the patients who received nivolumab as 2L/3L and regorafenib was the 2L in 86% of the patients who received nivolumab as 3L. Regarding the 18 patients treated with nivolumab as 2L (61.1% Child-Pugh A; 38.9% Child-Pugh B and 100% PS0-1), 5 discontinued the 1L due to AE without radiologic progression and the remaining presented BCLCp-B (16.7%), BCLCp-C1 (27.8%) and BCLCp-C2 (27.8%). In the nivolumab-2L cohort, median follow-up and OS since the start of 1L was 12.5 months (IQR 7.7-28.9) and 28.5 months (95%CI 15.5- 43.0) respectively. All, except 1 of 14 patients treated with nivolumab in the 3L (85.7% Child-Pugh A, 71.4% PS0 and 28.6% PS1) received nivolumab due to radiologic progression (BCLCp-B, C1 y C2: 7.1; 28.6 y 57.1%). In the nivolumab-3L cohort, median follow-up since 1L start was 21.3 months (IQR 15, 6-24, 4) and OS was not calculated owing to insufficient follow-up and number of events. Fifteen (46.8%) patients presented 25 AEs, of which 5 (20%) AEs were grade III-IV and 1 was grade V (rejection after liver transplantation). Corticosteroids were required for the management of AEs in 5 (15.6%) patients. There were 2 definitive discontinuations due to AEs (1 rejection after liver transplantation and 1 ascites).

Conclusion: The safety profile in this cohort is similar to that reported in clinical trials, despite the inclusion of patients in 2L and 3L. The heterogeneity in the patterns of progression before nivolumab and the fact that some patients started nivolumab due to intolerance to sorafenib/regorafenib without presenting radiologic progression highlights the need to consider these confounding factors when evaluating OS data.

P04-10YI Sulfatase-2 (SULF2) in the hepatocellular carcinoma microenvironment orchestrates disease progression and therapy resistance

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Background and aims: Sulfatase-2 (SULF2) is an endosulfatase that modifies sulfation of heparan sulfate proteoglycans. SULF2 expression is upregulated in a number of malignancies, including hepatocellular carcinoma (HCC). We previously showed SULF2 expression in HCC is more common in cancer associated fibroblasts (CAFs) than cancer cells, in association with a much poorer prognosis. We have explored the underlying mechanisms using Hep3B HCC cells, which do not express SULF2, in combination with LX-2 myofibroblasts, which do.

Method: Hep3B 3D hanging droplets were cultured in conditioned media (CM) from LX-2 or SULF2 knockdown (KD) LX-2 cells in the presence of inhibitors of selected oncogenic pathways or sorafenib (1.25-5.0 µM). Western blotting (WB) detected the levels of total or phospho-proteins. Stemness markers were assessed at mRNA and protein levels in tumour cells. SULF2, RelA-P-ser536, and CD44 tissue expression were explored by immunohistochemistry in HCC biopsies.

Results: Stromal-SULF2 induced growth of Hep3B spheroids, but this was abolished by an IKKβ inhibitor. WB confirmed that stromal SULF2 induced the phosphorylation of the RelA subunit of NF-κB, a downstream target of IKK. Sorafenib treatment had no impact on Hep3B spheroid growth, although its cytostatic effect was restored in spheroids grown in CM from SULF2 KD LX-2 cells. Notably, RelA phosphorylation persisted in Hep3B spheroids cultured in the presence of sorafenib in CM from LX2 cells, compared to suppression in SULF2 KD LX-2 CM alone ± sorafenib. CD44, a stem cell marker and a downstream target of NF-κB, was upregulated in Hep3B cells treated with control LX-2 CM ± sorafenib compared to tumour cells treated with SULF2 KD CM ± sorafenib. In a series of 20 patients with characterised SULF2 expression in biopsy tissues, nuclear RelA-P-ser536 and membranous CD44 positivity in tumour cells correlated with the presence of SULF2 in adjacent stromal cells (Spearman's Rho 0.776 p <0.001 and 0.744 p <0.001 respectively). In 9 patients treated with sorafenib, 7 had radiological progression of HCC after 3 months in association with SULF2 in CAFs and/or tumour cells in pre-treatment biopsies. In contrast, SULF2 expression was absent in 2/9 patients with stable disease up to 6 months (p = 0.028, Pearson Chi-Square).

Conclusion: CAF-derived SULF2 regulates the paracrine activation of the IKKβ/NF-κB/CD44 stemness pathway in neighbouring tumour cells, promoting sorafenib resistance.

P04-11 Analysis of epithelial to mesenchymal transition markers expression in liver of mice fed high fat diet

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Background and aims: The process of epithelial to mesenchymal transition (EMT) is a key element of metastasis in which epithelial cells acquire the features of mesenchymal cells facilitating their migration, invasion of surrounding tissues and metastasis. During EMT clearly polarized epithelial cells, with high expression of E-cadherin and other proteins characteristic for epithelial cells phenotype, under the influence of growth factors, cytokines, and other environmental factors change their morphology to an elongated one and become migrating cells with the expression of i.e. vimentin, fibronectin, N-cadherin. Loss of the expression of E-cadherin is a well-known sign of the EMT, which may cause the transformation of the non-malignant tumor into a locally aggressive and invasive form. Non-alcoholic fatty liver disease is defined as the accumulation of excessive fat in the liver in the absence of excessive drinking of alcohol and lack of any secondary cause. Although the disease remains asymptomatic most of the time, it can slowly progress from hepatic steatosis to hepatocellular carcinoma (HCC). Importantly, it was shown, that development and progression of HCC is tightly linked to EMT.
The aim of our study was to investigate the influence of high fat diet and fatty acids on the expression of EMT markers and evaluate the EMT process in murine primary hepatocytes and HepG2 cells.
Method: C57BL/6J mice were fed high-fat diet for 2-20 weeks, to reproduce features of the human NAFLD and next primary hepatocytes were isolated for further analysis. Experiments *in vitro* were performed using HepG2 cell line stimulated with 0.5 mM sodium oleate. Next, the level of mRNA and proteins were studied by real-time PCR and western blot. The location and distribution of EMT markers in cells was checked by immunofluorescence staining.
Results: Our study revealed that high fat diet increased the level of mesenchymal markers. We observed that prolonged high fat diet in mice (20 weeks) increased level of TGFβ, an important inducer of EMT. 20 weeks of diet increased also the level of transcription factors Zeb1, Zeb2 and Slug. We observed increased level of vimentin and β-catenin, characteristic for mesenchymal phenotype together with a decreased level of E-cadherin. Our observation was confirmed by *in vitro* data where the transcript level for mesenchymal markers also increased after treatment of HepG2 with 0.5 mM sodium oleate.
Conclusion: The results may contribute to a better understanding of the mechanism of EMT in liver cells.
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P04-12 CXCL5 induced by the synergy of transforming growth factor-beta and Axl signaling causes neutrophil extracellular trap formation in hepatocellular carcinoma

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Background and aims: Transforming growth factor (TGF)-beta and the receptor tyrosine kinase Axl that is activated by its ligand Gas6 are crucially involved in the development of hepatocellular carcinoma (HCC). Gas6/Axl interacts with 14-3-3-zeta to aberrantly activate TGF-beta/Smad3 linker region at serine 213 via c-Jun N-terminal kinase, causing the activation of pro-metastatic genes and autocrine TGF-beta signaling. In addition, the synergy of TGF-beta and Gas6/Axl induces the expression and secretion of CXCL5. Notably, CXCL5 is almost exclusively expressed in TGF-beta-positive HCC patients, correlating with neutrophil infiltration into the HCC microenvironment and poor patient survival. In this study, we aimed at investigating the TGF-beta/Axl/CXCL5-dependent attraction and activation of neutrophils.
Method: We employed human HCC models upregulating CXCL5 in response to long-term TGF-beta exposure. TCGA datasets of HCC patients (n = 400) were analyzed in tumor-, stroma- and immune-compartments by in silico microdissection. Formation of neutrophil extracellular traps (NETs) was studied by confocal immunofluorescence analysis and enzyme-linked immunosorbent assays by detecting citrullinated histone 3 (cit-H3) and neutrophil-specific enzymes. Inhibition of Axl and TGF-beta signaling in HCC cells was performed by pharmacological intervention, CRISPR/Cas9 genomic editing and RNA interference.
Results: The analysis of novel TCGA datasets revealed upregulation of CXCL5 in a large HCC patient cohort expressing Axl and TGF-beta. Loss of either Axl or TGF-beta signaling in human HCC cells abrogated CXCL5-dependent migration and attraction of neutrophils. Co-cultivation of CXCL5-secreting HCC cells with neutrophils induced NET formation as detected by the release of DNA associated with cit-H3 and neutrophil elastase as well as by the release of neutrophil DNA linked to myeloperoxidase. NET formation induced by the exposure of neutrophils to supernatants of CXCL5-positive HCC cells was diminished by treatment with neutralizing CXCL5 antibody. Pharmacological or genetic inhibition of either TGF-beta/Smad or Axl signaling reduced NET formation, suggesting that Axl/TGF-beta is essentially involved in regulating the immunophenotype. In HCC patient samples Axl, TGF-beta and CXCL5 expression correlated with neutrophil infiltration and NET formation.
Conclusion: The molecular collaboration between TGF-beta and Axl induces the CXCL5-dependent NET formation in HCC. Disruption of TGF-beta/Axl/CXCL5 signaling provides a promising therapeutic strategy to interfere with HCC progression in TGF-beta-positive patients.

P04-13YI The hepatocyte specific role of the NRF2/KEAP1 axis for HCC progression during chronic liver disease

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Background and aims: Hepatocellular carcinoma (HCC) occurs as a consequence of malignant transformation of hepatocytes frequently triggered during chronic inflammation and consecutive liver fibrosis. Oxidative stress has been considered as a conjoint pathological mechanism, and it contributes to initiation and progression of liver injury. The KEAP1 (Kelch-like ECH-associated protein-1)/NRF2 (erythroid 2-related factor 2) axis is a major regulator system of cellular redox balance. We investigated whether activation of the NRF2 pathway, by inactivation of the negative regulator KEAP1, affects the development of liver injury, fibrogenesis and HCC development.
Method: Hepatocyte specific NEMO (NEMO$^{\Delta hepa}$) knockout- mice were crossed with hepatocyte specific KEAP1 (KEAP1$^{\Delta hepa}$) knock-out mice to generate NEMO$^{\Delta hepa}$/KEAP1$^{\Delta hepa}$ mice. Primary hepatocytes as well as liver were subjected to microarray analysis. Furthermore, liver injury, DNA damage, proliferation as well as liver fibrogenesis and HCC development were analysed.
Results: Microarray analysis of primary hepatocytes of NEMO$^{\Delta hepa}$ and NEMO$^{\Delta hepa}$/KEAP1$^{\Delta hepa}$ and their controls revealed that particularly two signalling pathways were differentially regulated. Hepatocyte specific KEAP1 deletion upregulated genes involved in glutathione metabolism and xenobiotic stress (e.g. HO-2, Nqo1). Secondary, genes involved in cell cycle regulation and DNA replication were dramatically downregulated in NEMO$^{\Delta hepa}$/KEAP1$^{\Delta hepa}$ compared to NEMO$^{\Delta hepa}$ primary hepatocytes.
Accordingly, 8-week old NEMO$^{\Delta hepa}$/KEAP1$^{\Delta hepa}$ mice showed significantly elevated hepatic GSH levels and genes involved in the oxidative stress response compared to NEMO$^{\Delta hepa}$ mice. Furthermore, deletion of KEAP1 in NEMO$^{\Delta hepa}$ mice resulted in reduced apoptosis, proliferation and DNA damage. Subsequently, NEMO$^{\Delta hepa}$/KEAP1$^{\Delta hepa}$ mice displayed decreased fibrogenesis and in late disease stage a lower tumour incidence, a reduced tumour number and decreased tumour size.
Conclusion: Hepatocyte specific inactivation of KEAP1 in NEMO$^{\Delta hepa}$ livers attenuated apoptosis, DNA damage and hepatic fibrosis progression. Consequently, deletion of KEAP1 in NEMO$^{\Delta hepa}$ mice ameliorated HCC progression. Hence, KEAP1 is an attractive target to treat chronic liver disease.

P04-14YI Clinical validation of the role of contrast-enhanced ultrasound in the EASL guidelines for the diagnosis of hepatocellular carcinoma

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Background and aims: Contrast-enhanced ultrasound (CEUS) of liver nodules after an inconclusive CT/MRI was endorsed in the 2018 EASL guidelines for the management of hepatocellular carcinoma (HCC), but no validation of this flowchart exists to verify whether using CEUS as second diagnostic line is useful or a waste of resources.
Thus, this observational study aims to validate the role of CEUS in the diagnosis of HCC in real clinical practice.
Method: During a 6 months period, we prospectively enrolled patients at risk of HCC with liver nodules submitted to CEUS for the characterization of liver lesions. Exclusion criteria: CEUS performed to study treatment outcome, portal vein thrombosis or to choose target lesion, CT/MRI performed ≥3 months apart from CEUS. The theoretical impact of CEUS on the clinical decision was then assessed.
Results: A total of 43 patients (mean age 64y) with conditions at risk for HCC underwent CEUS to characterize 64 liver nodules (median diameter 19 mm, range 6-91 mm). A total of 12 lesions were excluded as not receiving CT/MRI within <3months from CEUS (none with a pattern of HCC). A total of 20 of the 52 remaining nodules received a diagnosis of HCC by CT/MRI. CEUS showed no case of LRM class (which would suggest a non-hepatocellular malignancy prompting biopsy), thus provided no additional benefit in this setting (in keeping with the guidelines). Conversely CEUS showed an HCC pattern in 4/32 (12.5%) nodules (4/16 patients, 25%) without a diagnosis of HCC at first CT/MRI. Of the remaining 28 cases one was LRM (and confirmed metastasis at histology), while no other showed the HCC pattern. Of these 4 typical HCC nodules 1 was diagnosed as such by biopsy, 1 by MRI after an inconclusive CT, 1 by repeating MRI in 2 months, 1 was not biopsied because of poor clinical conditions with ascites, but exams showed AFp >250.000 ng/ml.
Conclusion: The present observational prospective study validates the effectiveness of CEUS according to the diagnostic flowchart of the 2018 EASL guidelines for HCC. In particular using CEUS in second line after an inconclusive CT or MRI investigation was shown to be able to provide a correct diagnosis of HCC in 12.5% of cases missed by CT or MRI.

P04-15 Changes in the circulating miRNome profiles after therapy identify miRNA predictive biomarkers for HCC recurrence

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Background and aim: Hepatocellular Carcinoma (HCC) is the fifth cause of cancer-related death worldwide. The late diagnosis and the difficulty in predicting the evolution of this multifactorial pathology could explain the high mortality rate. Recently the potential role of microRNAs (miRNAs) as prognostic biomarker has witnessed an increasing interest owing to the non-invasive nature of miRNA-based screening assays. In this study, we used the weighted gene co-expression network analysis (WGCNA) to identify circulating miRNA clusters and miRNA candidates as predictive biomarkers for HCC recurrence.

Method: Twenty patients with early/intermediated stage HCC were enrolled and treated according to the EASL/AASLD practice guidelines. Patients were staged at time 0 and 1, 6, 12 month from therapy with CT scan and/or MRI the longest follow-up was 48 month. Blood samples were collected at the admission time and one month after the treatment. The miRNA 3.0 gene array (Affymetrix) was used to profile the whole blood circulating miRNome. The background normalized expression data were used for the WGCNA. Co-expressed miRNAs were included into the same module. Network modules were associated to the relevant clinical parameters and Hub miRNAs were selected as biomarker candidates.

Results: Fourteen co-expressed miRNA modules were identified: Among them one module negatively correlated with lower Disease Free Survival (DFS) (r = -52, p = 0.04), while a positive correlation was found for patients with a DFS>12 (r = 0.66, p = 0.03) in a second miRNA module. Within these modules, 3 hub miRNAs have a significant association with lower DFS: Mir-221-3p (r = -0.73, p = 0.01), let-7f-5p (r = 0.70, p 0.01), let-7a-5p (r = -0.67, p = 0.02). Nine hub miRNAs were significantly associated with DFS>12: MiR-4748 (r = 0.67, p 0.02), miR-486-3p (r = 0.63, p 0.04), miR-3688-3p (r = 0.61, p 0.05), miR-4755-5p (r = 0.82, p 0.002), miR-4742-5p (r = 0.71, p = 0.01), miR-3173-3p (r = 0.71, p = 0.02), miR-4708-5p (r = 0.64, p = 0.03), miR-4540 (r = 0.63, p = 0.04), miR-192-3p (r = 0.61, p = 0.04).

Conclusion: Network based approaches are promising tools to identify potential prognostic biomarkers in HCC.

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P04-16YI Acquisition of stem cell-like features in human cholangiocarcinoma is associated with an oxidative metabolism

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Background and aims: Cancer stem cells (CSC) are resistant to drugs and responsible for tumor initiation as well as relapse. However, mechanisms underlying CSC state in cholangiocarcinoma (CCA) remain largely unknown. Growing evidence indicated that deregulated cellular metabolism is linked to acquisition of tumor stem-like properties as well as drug resistance. In the present study we explored alterations of glucose metabolism in CCA stem-scenery.
role of CCL2 deficiency in a model of HCC associated with fatty liver.
Method: Stem-like compartment was enriched by sphere culture (SPH) in established human intrahepatic CCA cells (HUCCT1, CCLP1, SG231). CCA-SPH extracellular flux analysis was examined by seahorse technology. CCA-SPH expression of hexokinase II (HKII), pyruvate kinase M1 (PKM1), PKM2 and Peroxisome proliferator activated receptor gamma coactivator 1-α (PGC-1α) was investigated by western bottling. Metformin effect on survival was examined by MTT. Glucose uptake was quantified by incorporated (U-14C) deoxy-D-glucose.
Results: In contrast to cells grown as adherent monolayers (MON), metabolic analyses by seahorse technology revealed that CCA-SPH have a more efficient respiratory phenotype by mitochondrial oxidative phosphorylation (OXPHOS). In agreement, CCA-SPH cells showed down-regulation of the glycolytic marker HKII, indicating adaptation toward mitochondrial respiration. Also, PKM1 overexpression and PKM2 repression in CCA-SPH cells correlated with decrease of glucose uptake as well as with reduction of GLUT-1 expression. Finally, over-expression of PGC-1α in CCA-SPH indicated that mitochondrial biogenesis and respiration was functionally relevant in CCA stem-like cells. These data were corroborated by FACS analysis showing in CCA-SPH a higher mitochondrial membrane potential (MitoTracker Red) as well as elevated mitochondrial mass (MitoTracker Green). Consequently, CCA-SPH survival was impaired after targeting mitochondrial complex I (by administration of metformin, phenformin, rotenone), complex III (antimycin) or ATP sintase (oligomycin). More importantly, metformin drastically affected expression of CSC-like (CD13, CD133, EpCAM) and pluripotency genes (KLF4, NANOG, BMI1) as well as epithelial mesenchymal transition (EMT)-signaling (E-cadherin, Vimentin, ZEB1, ZEB2, Slug, Snail), suggesting its possible contribution to the CCA-stem subset. In an in vivo xenograft model, metformin limited the growth of CCA-SPH-derived tumors in immunocompromised mice, confirming the OXPHOS-associated phenotype of CCA-stem-like subset. Analysis of published microrarray-based data in 59 CCA vs. surrounding liver confirmed significant (FDR q-val <0.2) signals of stemness and OXPHOS.
Conclusion: Our findings indicate that CCA stem-like cells undergo a metabolic reprogramming resulting in OXPHOS addiction to meet energy demands.

P04-17YI Long-term survivors in patients with hepatocellular carcinoma: an ITA.LI.CA. report

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Background and aims: Hepatocellular carcinoma (HCC) is characterized by a poor prognosis but some patients reach a quite long survival, the predictors of the event not being fully elucidated. The aim of our study was to compare a long-term survival group (≥5 yrs) to a control group (<5 yrs) to identify parameters associated with good prognosis.
Method: To conduct this retrospective case-control study a group of HCC patients with survival ≥5 years (cases, n = 568) and a control group of equal size (n = 568) with survival <5 years were selected from the ITA.LI.CA. database, now including 6991 patients.
Results: Compared to controls, cases were less frequently cirrhotics (89.8% vs. 93%, p = 0.04) with less frequent portal hypertension (36.8% vs. 53.9%, p <0.0001) and had at diagnosis better residual liver function (Child A in 82.7% vs. 69.5%, p <0.0001). Moreover, they had their diagnosis more frequently during surveillance (80.1% vs. 71.4%, p = 0.0007), with less advanced HCC (in the majority of cases monofocal, whit a diameter ≤3 cm and in stage BCLC 0-A). The long-term survivors had been treated in 80.8% of cases with at least one curative therapy (resection or ablation) in their clinical history, against 56.6% of controls (p <0.0001). At a multiple logistic regression analysis, variables significantly associated with survival were: surveillance, ECOG-PS, portal hypertension, tumor aspect (monofocal/multifocal), diameter of the largest liver lesion, AFP levels and BCLC stage. The discriminant analysis identified diagnosis under surveillance, main treatment and BCLC stage as the variables with better discriminant capacity between the two groups, correctly classifying 77.2% of the cases, overall.
Conclusion: Preserved liver function, early stage at diagnosis and radical treatment are associated with good prognosis. Surveillance confirms its usefulness being in this study a sound predictive factor of long-term survival.

P05-01 Expression of galectin 3 and the clinical presentation of Hepatocellular Carcinoma recurrence after liver transplantation

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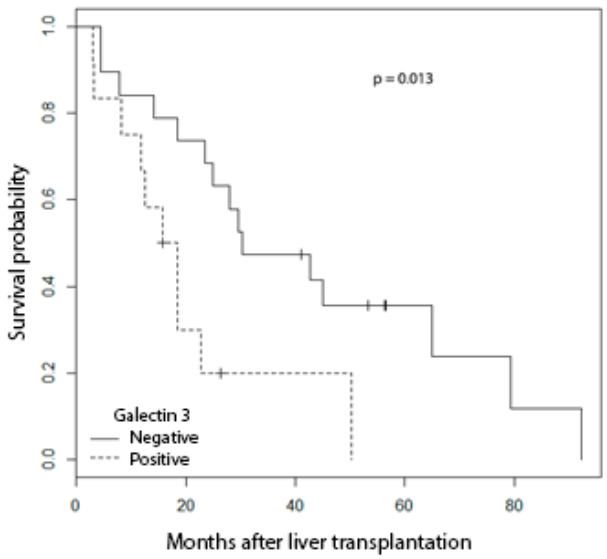
Background and aims: To describe the clinical outcome of patients who developed Hepatocellular Carcinoma (HCC) recurrence after liver transplantation and to analyze the impact of tissue expression of galectin 3 (Gal3) on the tumors' clinical presentation and the patients' survival.

Method: We analyzed a cohort of patients who developed HCC recurrence after transplantation at a referral centre in north-eastern Brazil. The patients were followed from the time of tumor recurrence (TR) until death. Tumor expression of Gal3 was determined by immunohistochemical analysis of the explant, and its association with TR characteristics and survival after transplantation was investigated.

Results: TR was observed in 36 of 212 patients with HCC that underwent liver transplantation. In 50% of these patients, TR was diagnosed within 1 year of transplantation. Only 5.6% of the patients presented with TR restricted to the graft and 50% had both hepatic and extra-hepatic involvement. The organs most often affected were the liver, lungs, and bones. An association was found between the tissue expression of Gal3 and early TR [odds ratio (OR), 24.4; 95% confidence interval (CI), 2.1-278.2; p <0.001] and the development of pulmonary metastasis (OR, 7.62; 95% CI, 1.15-50.5; p = 0.03). The median survival after transplantation was 24.9 months (95% CI, 18.4-50.3 months). An independent association was observed between the expression of Gal3 and post-transplant mortality due to TR (hazard ratio, 3.53; 95% CI, 1.24 -10.0; p = 0.018).

Conclusion: These results suggest that tissue expression of Gal3 may be associated with pulmonary metastasis and shorter survival in patients with post-transplant recurrent HCC.

Figure: Kaplan-Meier curves of post-transplant survival in patients with recurrent HCC, according to galectin3 expression (p log-rank = 0.013)



P05-02 Serum levels of protein induced vitamin K absence or antagonist-II (PIVKA-II) and alpha-fetoprotein (AFP) at baseline predict overall survival in patients with hepatocellular carcinoma (HCC)

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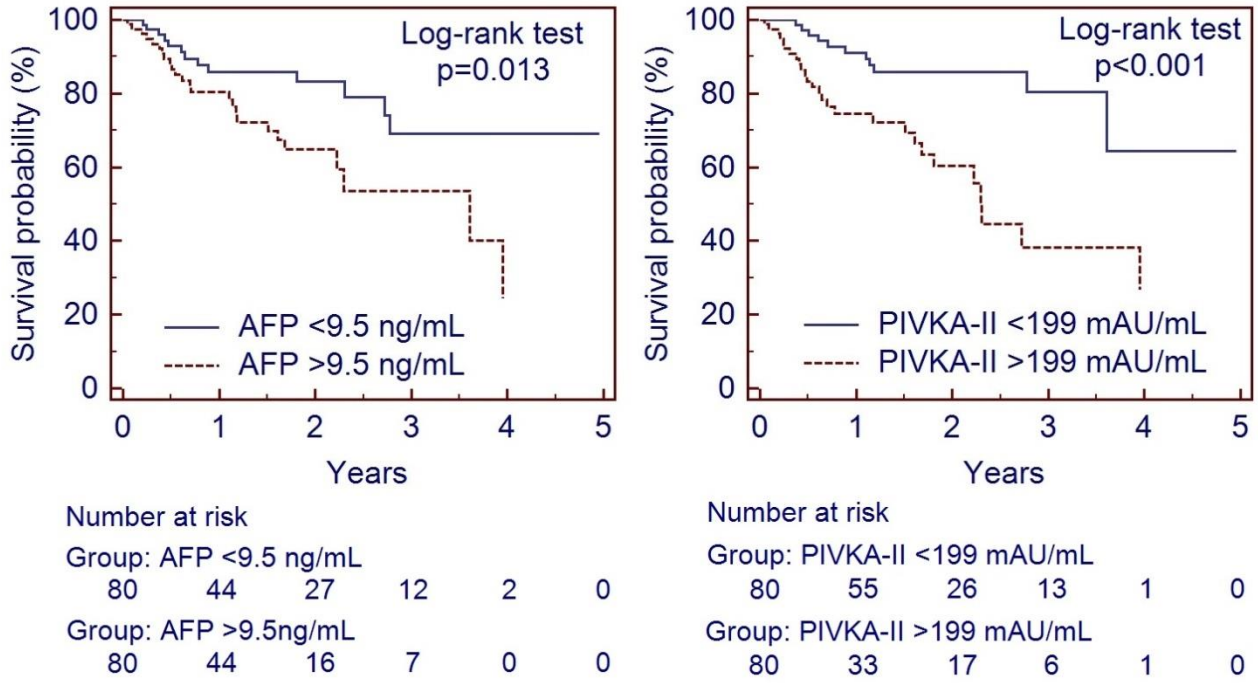
Background and aims: Non-invasive biomarkers of overall survival (OS) in patients with HCC are an unmet need. Our aim was to evaluate the prognostic value of AFP and PIVKA-II serum levels in patients with HCC.
Method: A total of 160 patients (28 F/132 M; median age: 65 [44-86] years) with a new diagnosis of HCC achieved between November 2012 and January 2018 were retrospectively analyzed. All patients had cirrhosis (Child-Pugh score: 119 A, 37 B and 4 C) and the main underlying etiology was viral (98 HCV, 20 HBV and 42 non-viral). HCC diagnosis was achieved by contrast-enhanced imaging or histological examination. Barcelona Clinic Liver Cancer (BCLC) staging system was adopted for patients classification (18 stage 0, 77 A, 39 B, 23 C, 3 D) according to EASL guidelines. Radiological response to treatment was assessed by modified Response Evaluation Criteria in Solid Tumors (mRECIST). Serum samples were collected at the time of HCC diagnosis. Serum AFP and PIVKA-II were measured by chemiluminescent enzyme immunoassays on Lumipulse® G600 II analyzer (Fujirebio, Japan).
Results: Median follow-up duration was 13.9 (95%CI 10.7-19.3) months. Therapy allocation followed BCLC staging. At the time of HCC diagnosis, median AFP and PIVKA-II serum levels were 9.5 (95%CI 7.0-15.1) ng/ml and 199 (95%CI 146-316) mAU/ml, respectively. Serum levels of PIVKA-II proportionally increased according to BCLC staging (p = 0.007), whereas AFP was not related to BCLC staging (p = 0.058). Both AFp >9.5 ng/ml and PIVKA-II >199 mAU/ml showed different survival curves (Fig. 1). Multivariate Cox-proportional hazard regression showed that AFp >9.5 ng/ml (HR = 2.41, 95%CI 1.17-4.97, p = 0.018), PIVKA-II >199 mAU/ml (HR = 2.90, 95%CI 1.35-6.19, p = 0.006), BCLC stage (HR = 1.94, 95%CI 1.35-2.78, p <0.001) and radiological response (HR = 0.13, 95%CI 0.04-0.40, p <0.001) were independent predictors of OS.
Conclusion: Higher serum levels of AFP and PIVKA-II were significantly associated with reduced survival independently from tumor stage and response to treatment. The determination of baseline serum AFP and PIVKA-II may be a useful prognostic factor in the management of patients with HCC.

Figure:

Figure 1



P05-03YI Molecular evaluation of skin proliferative lesions in patients with hepatocellular carcinoma under sorafenib treatment

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Background and aims: The onset of early dermatologic adverse events (eDAE) in patients with hepatocellular carcinoma (HCC) treated with sorafenib is associated with better prognosis. The most frequent DAEs are rash and hand-foot reaction. However, some patients may present proliferative skin lesions (SL) that require biopsy and/or surgical procedures, what affects treatment course and quality of life. According to pre-clinical models, sorafenib-induced inhibition of RAF triggers a paradoxical activation of MAPK pathway in keratinocytes that carry ultraviolet-induced mutations in genes such as HRAS. We aim to characterize the SL developed under sorafenib treatment, evaluate its impact on overall survival (OS) and determine mutations potentially associated with the pathogenesis of SL.

Method: A prospective database of patients with HCC treated with sorafenib was analyzed, and patients who developed SL submitted to pathologic examination were included. Biopsies were centrally reviewed and mutations in HRAS, KRAS and BRAF were determined by CAST-PCR.

Results: Between 2008 and 2018, 313 patients received sorafenib (54.6% BCLC C; 88.7% PS0 and 83.6% Child-Pugh A) and 89 (28.4%) presented eDAE. Median treatment duration and OS of the patients who presented eDAE were 6.7 months (3.5-15.9) and 18.2 months (95%CI 13.9-23.6), respectively. We identified 33 SL in 24 (7.6%) patients. The most frequent types were keratoacanthoma (n = 7; 21.2%), squamous-cell carcinoma (5; 15.2%), basal-cell carcinoma (3; 9.1%) and seborrheic keratosis (3; 9.1%). Median time to the onset of SL, treatment duration and OS of the patients with SL were 8.6 months (4.4-18.1), 12.5 months (9.5 a 22.0) and 26.5 months (95%CI 17.0- 43.9), respectively. Most (72.4%) of the proliferative SL showed lymphocytic infiltrate. HRAS mutations were identified in 1 squamous-cell carcinoma (G12D), 1 hypertrophic keratosis (Q61K and Q61L) and 1 basal-cell carcinoma (G12D). KRAS G12D was found in 1 basal-cell carcinoma and 1 sebaceous hyperplasia.

Conclusion: Proliferative skin lesions are late events during treatment and do not affect clinical outcomes. The association between sorafenib and skin malignancies stresses the need of dermatologic follow-up. Besides, the presence of lymphocytic infiltration reflects a potential immunomodulatory role of sorafenib, and the identification of mutations in genes implicated in the MAPK pathway suggests the mechanism of paradoxical activation.

P05-04 Safety and effectiveness of regorafenib in recurrent HCC after liver transplantation and progression on sorafenib: a real-life multicentre study

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Background and aims: Regorafenib improves the overall survival (OS) of sorafenib-tolerant patients who develop progression and it is the first second-line systemic treatment approved by EMA and FDA for hepatocellular carcinoma (HCC). However, there is no data of regorafenib in patients with HCC recurrence after liver transplantation (LT). Thus, the aim of this study is to evaluate the safety and outcomes of regorafenib in this population.

Method: This is a retrospective, multicentre and international study, including regorafenib-treated LT patients. The baseline characteristics and evolutionary events during sorafenib/regorafenib treatment were collected. Patients' management and radiological evaluations were performed according to centres' policy.

Results: From 2015 to 2018, 28 LT patients (57 years, 68% males, 54% performance status 1) from Europe and Latin-America were included. Median time from LT to regorafenib initiation was 3.9 (1.1-18.5) years, median time on sorafenib was 11.3 (0.7-76.4) months and from sorafenib discontinuation to regorafenib initiation was 14 (1-591) days. During regorafenib treatment (6.3 months), all patients had at least 1 adverse event (AE) of any grade, the most common grade 3/4 AEs were fatigue (25%) and dermatological reaction (18%). While no liver rejection episodes were observed, plasma levels of immunosuppressive drugs increased in 5 patients. Twenty-four patients developed radiological tumor progression: the most frequent patterns of progression were extra-hepatic growth (38%) and new extra-hepatic lesions/new vascular invasion (33%). Median OS from regorafenib initiation and sorafenib-regorafenib sequence were 12.9 (CI 95%; 6.7-19.1) and 38.4 (CI 95%; 18.5-58.4) months, respectively.

Conclusion: This is the first evidence that regorafenib is safe in patients after LT. The impact of sequential sorafenib-regorafenib treatment on OS in this population seems similar to the reported in no-LT patients.

P05-05YI Experience in the use of sorafenib impacts on reasons leading to discontinuation and chance of long term response

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Background and aims: Sorafenib is associated with various adverse events (AEs) potentially leading to permanent drug discontinuation. Common sense suggests that cumulative experience over a long timeframe might improve the management of drug-related AEs, with a potential benefit to the patients. However, the actual existence and the full extent of this phenomenon have never been investigated.
Method: We analyzed a large retrospective-prospective database gathering the clinical data of 201 patients from our Centre, who were consecutively prescribed with sorafenib between 2008 and 2017. We divided these patients in two groups according to the start date of sorafenib (2008-2012 vs 2013-2017), comparing clinical, laboratory and tumor characteristics. In particular, we verified: treatment duration, medium daily dose, reason of sorafenib discontinuation (as defined by Iavarone et al, Hepatology 2015), and overall survival (OS).
Results: One-hundred-three and 98 patients started sorafenib in 2008-2012 and 2013-2018, respectively. These groups did not differ in age, sex, performance status, liver function, and tumor staging. Due to more frequent dose reductions, the median average daily dose of sorafenib was lower in the 2013-2018 group (413 vs 518 mg/day, p <0.001). In parallel, the median treatment duration increased in the same group (145 vs 112 day, p = 0.027), with no remarkable difference in the cumulative drug dose between the two groups (61.6 vs 58.1 g, p = 0.440). The rate of patients permanently stopping sorafenib for intolerance dropped from 23.3% in 2008-2012 to 7.1% in 2013-2017 (p = 0.002). The median OS was similar in the two groups (11.1 vs 11.6 months), but the rate of long-survivors (OS>3 year) was higher in the 2013-2017 group (23.4 vs 9.7%, p = 0.001). To reduce the influence of deaths due to early progression, we performed a subgroup analysis of patients who achieved disease control as their best radiologic response. In this case, the OS in the 54 patients treated in 2013-2017 was significantly higher compared to that of the 51 patients treated in 2008-2012 (24.4 vs 20.6 months, HR 0.63, 95%CI 0.42-0.96, p = 0.031)
Conclusion: Increased experience in the management of sorafenib-related AEs may lead to increased treatment duration and better outcomes in sorafenib-responsive patients. This factor may be of paramount relevance in the era of sequential treatments based on tyrosine-kinase inhibitors, as these molecules share a common toxicity-profile.

P05-06 The current status of selecting treatment modalities for naiive hepatocellular carcinoma patients over 70 years old : Single-center experience

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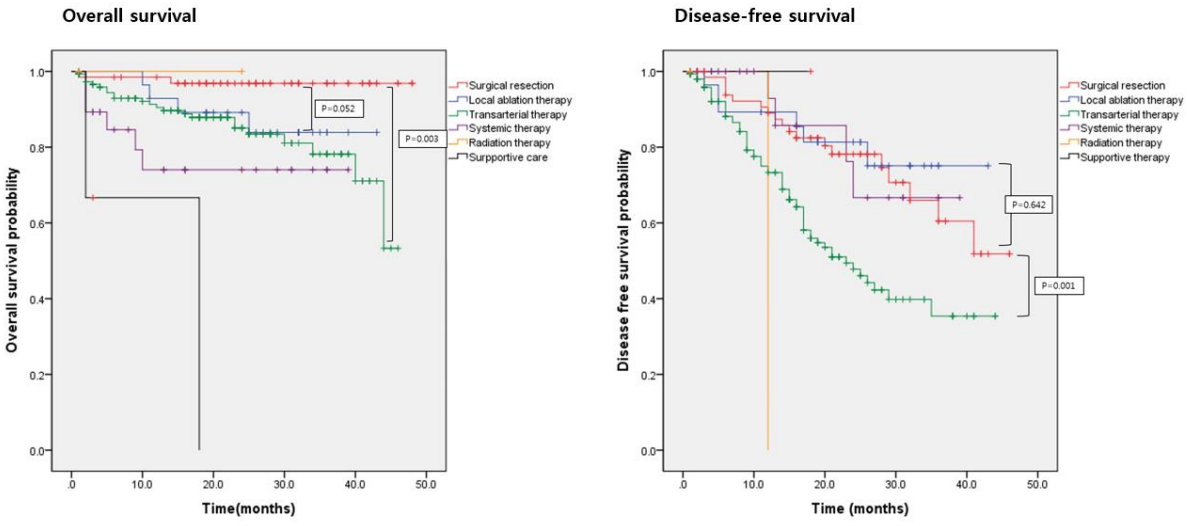
Background and aims: There are still some controversies on optimal therapeutic options for hepatocellular carcinoma (HCC) patients who were over 70 years old. This study evaluated the current status of treatment modalities for naiive elderly HCC patients in single-center to determine optimal management strategy.
Method: From January 2014 to December 2016, we reviewed medical records of 280 naiive HCC patients over 70 years old who visited the Hepatoma Clinic in Severance Hospital, Seoul, Korea. Clinicopathological data and survival analysis were analyzed.
Results: Among these patients, 39.3% of them were Hepatitis B carrier and 75% of them had been previously diagnosed as liver cirrhosis. More than half of the patients showed normal activity (ECOG scale 0), and 73.6% of the patients showed Child-Pugh score of 5 points. The mean MELD score of all patients was 8.1 ± 3.2, 64.6% was single lesion, and the mean preoperative radiologic tumor size was 4.6 cm. 65 of 280 patients (23.2%) underwent surgical resection who mostly considered as single lesion (95.4%), and all 65 patients obtained R0 resection. For the patients aged 70-75 years old, 26.9% of them underwent surgical resection, and 48.7% of them received transarterial therapy. For the patients over 80 years old, 6.7% of them underwent surgical resection, and 82.2% of them received transarterial therapy. There were statistically significant differences in selection of treatment between two groups. The overall survival and disease-free survival analysis showed a statistically significant superiority of surgical resection compared with transarterial therapy, but no statistical significance was observed compared surgical resection with local ablation therapy. Female, BMI, Child-Pugh score, MELD score, and tumor number were factors affecting the overall survival in the COX regression analysis. In the propensity score matching analysis of the surgical and transarterial therapy group, there was no significant difference between the two groups in overall survival and disease-free survival.
Conclusion: Although transarterial therapy is most common treatment of choice in elderly naiive HCC patients, adequate surgical resection is another important therapeutic option to consider in achieving survival benefit in treating HCC.
Figure:



P05-07YI fas and p53-upregulated modulator of apoptosis in hepatocellular carcinoma cellular death

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Background and aims: FAS/CD95 is the key component of the extrinsic death pathway that can induce apoptosis when binds to its cognate ligand FASL/CD95L. p53-upregulated modulator of apoptosis (PUMA) is a potent pro-apoptotic protein involves in p53-dependent and independent apoptosis pathway. However, whether PUMA play a role in the regulation of FAS in cancer is still unclear. This study reports on a relevant function of FAS and PUMA in hepatocellular carcinoma (HCC).
Method: For the *in vivo* study, the mRNA analysis of FAS, FASL, and PUMA were collected from 111 patients undergoing liver resection without any prior treatments (39 HCC, 30 peri-HCC, 31 distal/cirrhosis); liver donors were used as control (11 normal). For the *in vitro* study, human immortalized hepatocyte cell line IHH and HCC cell line HepG2 were used. Apoptosis-induction was performed by using anti-FAS (DX2) at concentrations of 250 ng/ml and 500 ng/ml for 24 hours. Flow cytometry was performed for FAS/FASL positivity and Annexin-V- PI apoptosis test, quantitative real-time PCR for mRNA expressions, and growth curve test for cells viability.
Results: The expressions of FAS and FASL mRNA were significantly increased in HCC as compared to normal tissues ($p < 0.05$). PUMA mRNA was positive in 13/32 (41%) HCC tissue samples, 19/27 (70%) in peri-HCC and 24/31 (77%) cirrhosis tissues samples. For *in vitro* study, anti-FAS treatment did not affect cells viability up to 13 days. After 24 hours treatment, PUMA mRNA was up regulated only in hepatocyte cell line where the transcription factors p53 and c-Myc were up-regulated. In contrast, all these transcription factors were down-regulated in HCC cell line.
Conclusion: HCC might have resistant mechanisms against FAS-dependent apoptosis. Deficiency of PUMA in HCC indicates its important function in apoptosis pathway. Deregulation of FAS and PUMA are involved in HCC development.

P05-08 Clinical safety, tolerability and adverse events of special interest in a Phase Ib study of atezolizumab (atezo) + bevacizumab (bev) in hepatocellular carcinoma (HCC)

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Background and aims: Agents targeting PD-L1/PD-1 signalling or angiogenesis are approved treatments (tx) for patients (pts) with advanced HCC. In this Phase Ib study (NCT02715531), combining atezo (anti-PD-L1) and bev (anti-VEGF) showed promising clinical activity, including investigator-assessed RECIST v1.1 confirmed ORR of 32%, median PFS of 14.9 mo, and DOR range of 1.6+ to 22.0+ mo in 73 efficacy-evaluable pts (follow-up [f/u] ≥16 wk; median f/u, 7.2 mo; Pishvaian, ESMO 2018). We present safety data focused on key adverse events (AEs).

Method: Pts with unresectable or advanced HCC with Child-Pugh score of A or B7 received atezo 1200 mg + bev 15 mg/kg IV q3w as first-line tx until disease progression (PD), loss of clinical benefit or unacceptable toxicity. Primary end points included safety.

Results: As of 26 July 2018, 103 pts were evaluable for safety, with median tx durations of 3.5 mo (range, 0-24 mo) and 3.5 mo (range, 0-23 mo) for atezo and bev, respectively. All-cause any-grade (Gr) AEs occurred in 95 pts (92%). Gr 3/4 AEs were seen in 41 pts (40%), Gr 5 AEs in 5 pts (5%). 36 pts (35%) experienced serious AEs (SAE); in 19 pts (18%) these SAEs were tx-related. AEs leading to discontinuation of any tx occurred in 11 pts (11%). All AEs leading to any atezo and/or bev withdrawal were single events, except oesophageal varices haemorrhage (n = 2 [2%], leading to bev discontinuation only). See table for additional safety data.

Conclusion: In general, the combination of atezo + bev was well tolerated and observed toxicities were manageable. No new safety signals were identified beyond the established safety profile for each individual agent.

Table:

AEs, n (%)	Safety-Evaluable Population (n = 103)
Any-Gr TRAEs	84 (82)
Gr 3/4	28 (27)
Gr 5[a]	2 (2)
Atezo any-Gr AESIs	56 (54)
Requiring systemic corticosteroids	12 (12)
≥5% prevalence	
Rash	21 (20)
AST increased	13 (13)
Blood bilirubin increased	11 (11)
ALT increased	8 (8)
Any-Gr hepatic events	30 (29)
Gr 3/4	17 (17)
Gr 5[b]	1 (1)
Bev any-Gr AESIs	48 (47)
≥5% prevalence	
Proteinuria	20 (19)
Hypertension	17 (17)
Epistaxis	10 (10)
GI bleeding[c]	6 (6)
Any-Gr bleeding events	24 (23)
Gr 3/4[d]	6 (6)
Gr 5[e]	1 (1)

AESI, adverse event of special interest; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GI, gastrointestinal; TRAE, treatment-related adverse event.
[a] Hepatic cirrhosis (1) and pneumonitis (1).
[b] Hepatic cirrhosis in a pt with baseline Child-Pugh score of B7, related to atezo and bev.
[c] Includes reports of oesophageal varices haemorrhage (2), upper GI haemorrhage (2), GI haemorrhage (1) and gastric haemorrhage (1).
[d] No Gr 4 AEs. Gr 3 AEs included oesophageal varices haemorrhage (2), upper GI haemorrhage (1), GI haemorrhage (1), intra-abdominal haemorrhage (1) and subarachnoid haemorrhage (1).
[e] Haemorrhagic shock after PD with portal vein thrombus, unrelated to tx.

P05-09YI cRel is a novel tumour suppressor in Hepatocellular Carcinoma

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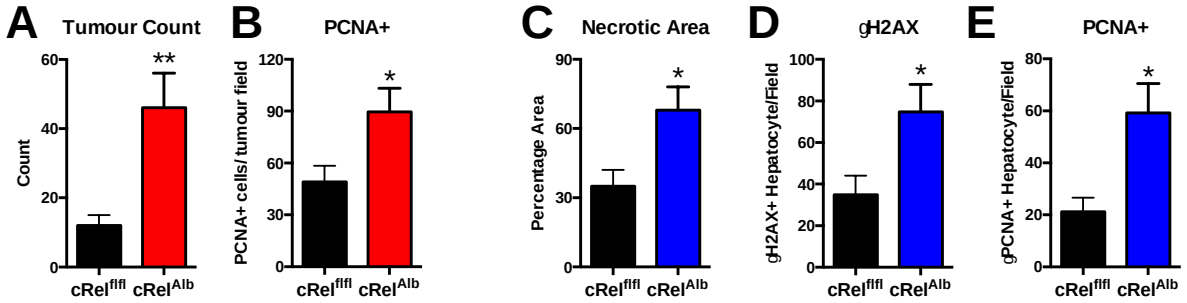
Background and aims: Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and is a leading cause of cancer-related death worldwide. The cRel subunit of the transcription factor NF-κB has been implicated in cancer and is widely considered to promote tumourigenesis. However, recently it has been described that cRel acts as a tumour suppressor in a murine model of B-cell lymphoma. We wish to discern whether cRel plays a tumour suppressive role in the development of liver cancer as well as understand the specific cellular mechanisms underpinning the tumour suppressive role of cRel in the development of cancer.
Method: Wild type (WT) and global cRel$^{-/-}$ mice were utilised as well as epithelial specific cRel knockout mice (cRel^{Alb}), generated by crossing Alb-cre mice with cRel$^{fl/fl}$ mice. The 30-week N-Nitrosodiethylamine (DEN) model was used to induce hepatocellular carcinoma (HCC) in global cRel$^{-/-}$, WT and cRel^{Alb} mice. An acute DEN injury model consisting of a single intraperiteonal injection of 100mg/kg DEN was used to assess hepatic responses to genotoxic injury in global cRel$^{-/-}$, cRel^{Alb} and control mice. Primary murine hepatocytes were isolated from global WT and cRel$^{-/-}$.
Results: Global cRel$^{-/-}$ mice develop more tumours than WT controls 30 weeks post DEN. The cell specific tumour suppressive role of cRel in the hepatocyte was confirmed in cRel^{Alb} mice which exhibited a significant increase in tumour number and tumour stage compared to control mice **(Figure A)**. cRel deficient tumours were also larger and more proliferative at 30 weeks post DEN **(Figure B)**. Underpinning this increase in tumour burden was an increase in cell death as a result of genotoxic injury with, global cRel$^{-/-}$ mice and cRel^{Alb} mice having increased liver damage, inflammation and compensatory proliferation following an acute dose of DEN **(Figure C-E)**.
Interestingly, primary murine hepatocytes isolated from global cRel$^{-/-}$ were more susceptible to both DEN and ionising radiation-induced DNA damage, compared with WTs. Moreover, we show, for the first time, that cRel is a critical regulator of the ATM-CHK2 DNA damage response *in vivo* following genotoxic injury. We propose that disruption of this pathway in cRel$^{-/-}$ mice drives genomic instability and tumourigenesis.
Conclusion: Our data provides the first evidence of cRel acting as a tumour suppressor in a solid organ tumour. An increased cancer risk should therefore be taken into consideration when developing treatments directed towards cRel in the future.



P05-10YI The development of hepatocellular carcinoma is not predicted by genetic variants in caucasian compensated HBV cirrhotics treated by Entecavir of Tenofovir for 10 years

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Background and aims: Signal transducers and activators of transcription (STAT4) genetic variant is associated with greater hepatic inflammation and fibrosis in untreated HBV Caucasian, while 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) to the development of hepatocellular carcinoma (HCC) in untreated or IFN-treated Asian HBV patients. Aim was to assess whether these genetic variants predict the HCC onset also in Caucasian HBV cirrhotics long-term treated by Tenofovir (TDF) or Entecavir (ETV).
Method: 258 Caucasian HBV-monoinfected HCC-free CPT-A cirrhotics were consecutively enrolled in a longitudinal cohort study at the TDF/ETV introduction. Excluded were HCC developed within the first year. At baseline: age 61 (21-83) year, 82% males, 88% HBeAg negative, 69% with normal ALT, 60% NUCs-experienced, BMI 25 (17-40) kg/m^2, 12% diabetics, spleen length 11 (7-20) cm, 14% with esophageal varices. Regular six-months blood tests and abdominal imaging were performed, until HCC diagnosis or Nov 2018. Seven SNPs were genotyped by using end point method with TaqMan probes: STAT4 *rs7574865*, EGF1 *rs4444903,* TLL1 *rs17047200,* MERTK *rs4374383,* MERTK2 *rs672663*, PNPLA3 *rs738409* and MBOAT7 *rs641738*.
Results: During 115 (20-150) months of TDF/ETV treatment, 41 (16%) patients developed an HCC after 53 (18-119) months. The 10-year cumulative HCC incidence was 20% (yearly rate 2.2%). 28% of the enrolled population has STAT4 GT/TT genotype, 18% EGF GG, 26% TLL1 AT/TT, 19% MERTK AA, 19% MERTK2 AA, 10% PNPLA3 GG, and 19% MBOAT7 TT. The 10-year cumulative incidence of HCC was similar across different genotypes: 26% vs 17.5% for STAT4 (GT/TT vs GG, p = 0.158), 10% vs 22% for EGF (GG vs CG/CC, p = 0.138), 18.7% vs 19.8% for TLL1 (AT/TT vs AA, p = 0.729), 15.7% vs 20.4% for MERTK (AA vs AG/GG, p = 0.783), 16.5% vs 20.4% for MERTK2 (AA vs AG/GG, p = 0.859), 19.2% vs 19.5% for PNPLA3 (GG vs CG/CC, p = 0.995), 25.7% vs 17.7% for MBOAT7 (TT vs CT/CC, p = 0.598). The only independent baseline predictors of HCC were spleen length (HR 1.33, 95%CI 1.1-1.5, p <0.001) and age (HR 1.09, 95%CI 1.0-1.1, p <0.001).
Conclusion: In Caucasian HBV compensated cirrhotics NUC-treated for 10 years, older age and severity of portal hypertension, but not genetic variants, predict the development of HCC.

P05-11 Impact of screening in the average survival time of cirrhotic patients with HCC

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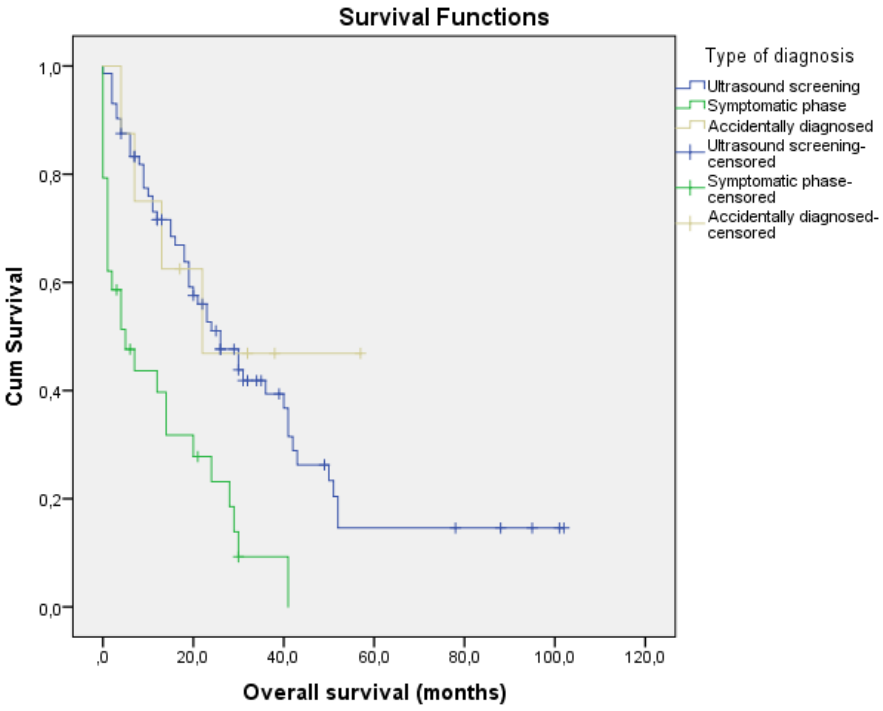
Background and aims: Hepatocellular carcinoma (HCC) represents one of the most feared complication of liver cirrhosis. It is currently recommended to perform an ultrasound screening every six months in all patients with cirrhosis in order to detect the tumour at an early and potentially curable stage. Our aim was to characterise the population of cirrhotic patients diagnosed with HCC and the impact of routine screening for HCC on the average survival time of these patients.
Method: A retrospective and unicentric cohort study including patients with cirrhosis in whom HCC was diagnosed between 2009 and 2017.
Results: A total of 109 patients were included, mainly males (83.5%). Mean age was 64.3 ± 12.2 years. The most frequent aetiologies of cirrhosis were alcohol (37.3%) and HCV infection (32.7%). Child-Pugh class distribution: class A in 66.1%, B in 24.8% and C in 9.2%. In addition to cirrhosis, one risk factor for HCC was identified in 45% of patients, two risk factors in 5.5% and no other risk factors identified in 49.5%.
Regarding the diagnosis of HCC, the majority was diagnosed during ultrasound screening (66.1%), 26.6% were diagnosed after appearance of symptoms and 7.3% diagnosed by chance during another investigation. Concerning the Barcelona Clinic Liver Cancer Classification (BCLC) score at time of diagnosis, 33.9% were BCLC A, 33% were BCLC B, 23.9% were BCLC C and 9.2% BCLC D.
Mean overall survival (OS) was 30.4 ± 3.5 months. Seventy-seven patients died (70.6%). According to the diagnostic method, the group of patients diagnosed with HCC during ultrasound screening had the longest mean survival time (35.5 ± 4.4 months), followed by those who were accidentally diagnosed (33.2 ± 8.4 months). Patients diagnosed with HCC after appearance of symptoms had the lowest mean survival time (12.3 ± 2.7 months). In multivariate analysis, being under routine screening for HCC was statistically associated to overall survival with an odd ratio of 0.557 (95% CI: 0.336-0.922, p value = 0.023).
Conclusion: This study emphasizes the importance of routine screening for HCC in patients with cirrhosis and its statistically significant impact on overall survival.
Figure:



P05-12 Serum metabolites as diagnostic biomarkers for cholangiocarcinoma, hepatocellular carcinoma and primary sclerosing cholangitis

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Background and aims: Early and differential diagnosis of intrahepatic cholangiocarcinoma (iCCA) and hepatocellular carcinoma (HCC) by non-invasive methods represents a current clinical challenge. The analysis of low-molecular weight metabolites by new high-throughput techniques is a novel strategy for identifying biomarkers. Here, we have investigated whether serum metabolome can provide useful biomarkers in the diagnosis of iCCA and HCC and could discriminate iCCA from HCC. Since primary sclerosing cholangitis (PSC) is a risk factor for CCA, serum metabolomic profiles of PSC and CCA have also been compared.

Method: Chloroform/methanol and methanol extracts obtained from the serum of patients with PSC, iCCA, or HCC and healthy individuals were analyzed using ultra-performance liquid chromatography coupled to mass spectrometry (UHPLC-MS).

Results: The analysis of the levels of lipids and amino acids in the serum of patients with iCCA, HCC, PSC and healthy individuals (n = 20/group) showed differential profiles. Several metabolites presented high diagnostic value for iCCA vs control, HCC vs control, and PSC vs control, with areas under the receiver operating characteristic curve (AUC) greater than those found in serum for the non-specific tumor markers carbohydrate antigen 19-9 (CA19-9) and alpha-fetoprotein (AFP), commonly used to help in the diagnosis of iCCA and HCC, respectively. The development of an algorithm combining glycine, aspartic acid, SM (42:3) and SM (43:2) permitted to accurately differentiate in the diagnosis of both types of tumors (biopsy-proven). The proposed model yielded 0.890 AUC, 75% sensitivity and 90% specificity. Another algorithm by combination of PC (34:3) and histidine accurately permitted to differentiate PSC from iCCA, with an AUC of 0.990, 100% sensitivity and 70% specificity. These results were validated in independent cohorts of 14-15 patients per group and compared with profiles found in NAFLD/NASH patients.

Conclusion: Specific changes in serum concentrations of certain metabolites are useful to differentiate iCCA from HCC or PSC, and could help in the early diagnosis of these diseases.

P05-13 Imaging and Clinical Features of HCC nidus: A Retrospective Study

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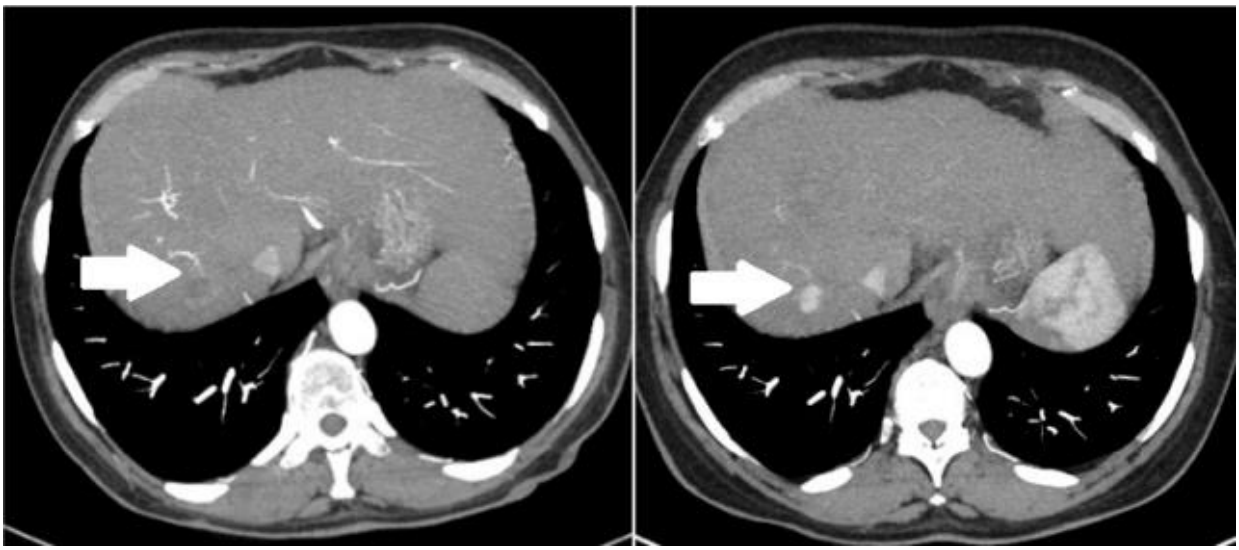
Background and aims: To describe specific imaging findings of early hepatocellular carcinoma (HCC) in correlation with clinical parameters

Method: Retrospectively, we reviewed 212 patients with HCC that had a previous imaging study by the time of a perceptible lesion less than 20 mm in size (HCC nidus), and at least one follow-up study after the diagnosis. All clinical data was collected referring to the date of the studies. The study was approved by the ethics committee of the institution

Results: Of the total, 42 patients (102 studies) met the inclusion criteria and did not have any type of procedure. 26 were males (61.9%) and 16 were females (38.1%); 32 patients were white (76%), 8 were black (19%) and 2 had other race (4.7%). The cirrhosis type most prevalent was hepatitis C (HCV) cirrhosis with 24 cases (57.1%), followed by alcoholic cirrhosis (EtOH) (16.6%), NASH disease (9.5%) and combined EtOH and HCV cirrhosis (4.7%). Segment VIII showed to be the most prevalent site of HCC nidus (26.1%). At the time of a perceptible nidus, AFP level above 20ng/ml was only seen in 3 patients and the overall mean was 9.24ng/ml; a perceptible unpaired hepatic artery supplying the HCC nidus was found in 26.1% of the cases and a capsular artery supply in 3 cases (2.3%). The mean tumor volume doubling time was 35 months with a range of 1.5 to 842.3 months. Platelet count mean by the time of nidus was 100.4 per ml on the HCV group, contrasting with 64.6 per ml in the ethanol group and 52.2 per ml in other cirrhosis types group (p = 0.015). AFP mean was also greater prior diagnosis in the HCV group (115.9ng/ml) contrasting with the EtOH group
(2.37ng/ml) and other types of cirrhosis group (5.8ng/ml) (p = 0.0057). Platelet count mean at the time of diagnosis was 95 per ml in the HCV group, 82.6 in the EtOH group and 93 in the Other types group. The AFP levels mean by the time of diagnosis 74.9 for the HCV group, 214.5 at EtOH group and 7.8 at Other types group.

Conclusion: This study reveals the presence and characteristics of an evolving HCC when the AFP levels are still normal. Platelet count mean and AFP levels mean are significantly greater in the HCV cirrhosis with HCC patients. This reinforces the difference of etiologies of HCC and the role of imaging for earliest detection and undisputed need for screening to provide patients with the best care. A cohort of cirrhotics without HCC, matched for appropriate clinical parameters, will be included in our final analysis.

Figure:



P05-15YI Circulating neutrophils present a dysfunctional immunophenotype in patients with hepatocellular carcinoma

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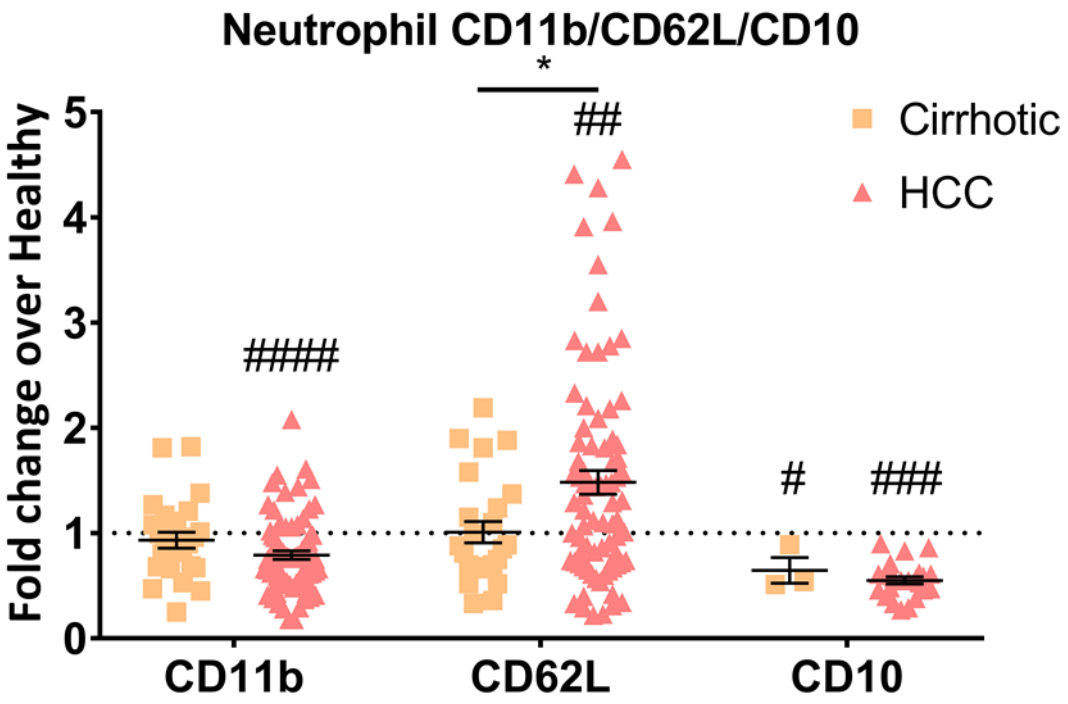
Background and aims: Chronic inflammation is a common feature underpinning the development/progression of 90% of hepatocellular carcinomas (HCC). Neutrophils have emerged as key immune mediators involved in promoting cancer progression/metastases. Our group has previously demonstrated that neutrophil depletion in a murine HCC model has profound anti-tumour effects. Furthermore, the peripheral circulating neutrophil count was an independent predictor of patient outcome. Pan-neutrophil depletion is not a viable option for cancer patients already susceptible to infection. However, distinctive cancer-specific markers and functions are still poorly defined. Here we aimed at exploring different immunophenotypic features of peripheral blood neutrophils.
Method: Neutrophil heterogeneity was assessed between liver cirrhosis and HCC patients (Newcastle, 2016-2018), together with healthy volunteers (n = 25, 81, and 47, respectively), by flow cytometry using FSC/SSC and the cell surface markers CD11b, CD62L, HLA-DR, CXCR4, CD15, CD16, c-MET, PD-L1, TIM-3 and CD10. Neutrophil function was assessed by measuring levels of reactive oxygen species (ROS) basally and in response to N-formyl-Met-Leu-Phe (fMLP) and platelet-activating factor (PAF).
Results: Circulating neutrophils from cancer patients presented a significantly impaired oxidative burst compared to healthy volunteers and cirrhotic patients following stimulation with fMLP and PAF (1650.681 ± 105.908 vs 2037.609 ± 218.219 and 2206.174 ± 227.951, respectively; $p < 0.05$). When analysing the expression of several surface markers by flow cytometry as fold-change over healthy controls, it was observed that neutrophils from HCC patients also had significantly lower levels of CD11b (0.790 ± 0.041; $p < 0.0001$), and higher levels of CD62L when compared to healthy volunteers and cirrhotic patients (1.482 ± 0.113; $p < 0.01$ and $p < 0.05$, respectively). Other markers investigated remained unchanged, apart from c-MET and PD-L1, where cancer patients had significantly increased percentages of neutrophils positive for these compared to healthy controls (c-MET 46.32 ± 4.159 vs 22.14 ± 3.447, $p < 0.01$; PD-L1 95.12 ± 0.822 vs 87.28 ± 3.219, $p < 0.01$), and CD10, with both cirrhotic and cancer patients presenting lower levels of this maturity marker (0.647 ± 0.122, $p < 0.05$, and 0.550 ± 0.035, $p < 0.0001$, respectively). Conditions for optimal neutrophil proteomics have been achieved, and a comparison of the proteomic profile between non-cirrhotic, cirrhotic and HCC neutrophils will be presented.
Conclusion: This data suggests that there are phenotypic and biologically relevant changes in peripheral blood neutrophils from HCC patients. Further characterisation of these cells may enable us to better define the “pro-tumour neutrophils” associated with HCC and develop potential tailored therapeutics to target these cells selectively.

Figure:


Neutrophil CD11b/CD62L/CD10
Fold change over Healthy
0
1
2
3
4
5
*
##
####
#
###
Cirrhotic
HCC
CD11b
CD62L
CD10

P05-17YI impact of interval between complete ablation of hepatocellular carcinoma and start of direct acting antivirals to manage chronic hepatitis C infection

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Background and aims: During these recent years, studies focused on the relation between the incidence and recurrence of hepatocellular carcinoma (HCC) and the use of direct acting antivirals (DAAs) to manage hepatitis C virus (HCV). In our study, we looked for the impact of time interval between complete HCC ablation and the start of DAAs in HCV-related HCC patients in terms of survival rates, tumor recurrence and response to DAA therapy.
Method: We included 60 HCC patients who presented to our HCC multidisciplinary clinic (Cairo University, Egypt) and planned to receive DAA therapy. They were completely HCC ablated before DAA treatment. They were followed up post DAA and we compared between patients who took DAA therapy less than and more than 6 months after HCC ablation.
Results: Among the 60 studied patients, 48 (80%) were males and the mean age was 57.87 ± 7.23 years. The lesions were predominantly single (65%), located in the right lobe (76.7%) and their mean size 3.56 ± 1.86 cm. Majority of patients were Child Pugh A (86.7%) and the remaining were Child B7. Fifty-two patients (86.7%) had complete ablation after initial modality of ablation while 8 patients (13.3%) needed further treatment to achieve complete response. Rate of HCC recurrence post DAAs was 45% with a mean duration between initial ablation and passing through follow-up before DAA treatment and ending by appearance of recurrence 14.88 ± 7.23 months. The mean survival duration is 34.23 ± 16.16 months. Patients who were given DAAs before elapse of six months after complete ablation (36 patients) had statistically significant worse survival (36.1%) than patients who waited for more than six months before starting DAAs (24 patients) (83.3%). No such difference was detected in terms of HCC recurrence or response to DAA therapy.
Conclusion: Increasing the duration between complete HCC ablation and start of DAA therapy to more than six months provides better survival rates. No such impact was observed as regards rates of HCC recurrence or response to DAA therapy.
Figure:

		Duration between DAA and ablation				
		<6 months (36)		>6months (24)		P value
		Count	%	Count	%	
HCC recurrence		19	52.8	8	33.3	0.138
Survival	alive	13	36.1	20	83.3	<0.001
	Death	23	63.9	4	16.7	
Response to DAA	Responder	33	91.7	20	83.3	0.280
	Relapse	3	8.3	2	8.3	
	Resistant	0	.0	2	8.3	

P06-01YI Lymphocytes count predicts the evolution of patients with hepatocellular carcinoma treated with sorafenib

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Background and aims: Sorafenib is a multi-kinase inhibitor with anti-angiogenic and anti-proliferative activity, but immunomodulation has also been hypothesized as a mechanism of action. Previous data suggest that neutrophils-to-lymphocytes ratio (NLR) is a prognostic factor in HCC. We aim to evaluate the prognostic role of lymphocytes (L), neutrophils (N) and platelets (P) counts as well as NLR and platelet/lymphocyte ratio (PLR) in HCC patients treated with sorafenib.

Method: Baseline and evolutionary variables were recorded from a prospective database of patients treated with sorafenib between 2008 and 2018. Clinical and laboratorial assessments were performed monthly and imaging follow-up was performed at the baseline, at month-1 and every 2 months thereafter. Treatment was discontinued in case of symptomatic progression, limiting toxicity, 2nd line start or death. Overall survival (OS) was estimated by Kaplan Meier method, compared by log-rank and adjusted with a Cox regression model. The patients were grouped into “high” and “low” according to the lower limit of normality of L, N and P; and into “high” or “low” NLR and PLR according to the median.

Results: 306 patients were included (83.3% male, 83.6% Child-Pugh A and 54.3% BCLC C). Median OS of the entire cohort was 13.6 months (CI95% 11.8-14.9). Performance-status, BCLC stage, L count and NLR were independent baseline prognostic factors ($p = 0.01$; $p = 0.04$; $p = 0.04$ and $p < 0.0001$, respectively). In a time-dependent analysis, high L was associated with better prognosis (HR 0.6; 95%CI 0.5-0.7) whereas high NLR was associated with worse prognosis (HR 1.8, 95%CI 1.4-2.3). The subgroup of patients who presented high baseline L and sustained high L during the first month (n = 169) presented better OS comparing to those with low L during the first month (n = 68) (15.9 vs 9.1 months; HR 0.5; 95%CI 0.4-0.5; $p < 0.0001$). Additionally, those patients with high NLR at baseline and during first month (n = 101) had median OS significantly shorter than those whose NLR behave low during the first month (n = 118) (10.1 vs 17.5 months; HR 2.1 95%CI 1.5-2.8; $p < 0.0001$). N, P and PLR were not independent prognostic factors.

Conclusion: Our study validates the prognostic role of NLR. However, the absence of a reference of normality of NLR limits its applicability. On the other hand, we demonstrated that L acts as a baseline and evolutionary prognostic biomarker and can be applied as an early marker of better treatment outcome in HCC patients treated with sorafenib.

P06-02YI HCC recurrence after DAA treatment in HCV patients.

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Background and aims: The present real-life multicenter, prospective study aims to investigate the effects of DAAs IFN-free therapies in HCV patients with a previous successfully treated HCC, in terms of neoplastic recurrence and SVR rates.

Method: From March 2015 to March 2017, all consecutive HCV patients with a previous successfully treated HCC and underwent to DAAs therapy were enrolled. The baseline clinical, biochemical and radiological data were registered. The assessment of neoplastic recurrence was used as primary outcome, while a secondary outcome was the evaluation of patients characteristics predicting HCC recurrence. Cumulative probabilities of recurrence were extracted from time-to-event curves based on Kaplan-Meier product limit method Hazard ratios (HRs) with 95% confidence intervals (95% CIs) were estimated using univariate and multivariable Cox regressions.

Results: A total of 101 patients were enrolled in the defined time period. The majority of patients were males (65/101, 64.4%), the mean age was 67.4 ± 7.7 years. Eighty-three percent of patients were in Child-Pugh class A, and 89% had a history of HCC BCLC stage 0/A, while 35% of patients had a prior HCC recurrence. Ninety-one percent of the patients achieved SVR. The median time between the HCC 1st diagnosis and DAAs-starting was 17.2 months (range 10.1-37.2), while the median time from the last successful HCC treatment to DAAs starting was 10 months (range 5.8-16.6). Thirty-one HCC recurrences were observed from DAAs-starting in a median observational follow-up of 31.7 months. The incidence rate of recurrence was 20.5/100 person-year (95% C.I. 13.9-29.0). The 6-, 12- and 24-months HCC recurrence rates from the last HCC treatment were 1%, 8.9% and 25.6%, respectively. Higher BMI (HR 1.20, 95% C.I. 1.01-1.42, p = 0.020), higher levels of Total Bilirubin (HR 2.54, 95% C.I. 1.04 to 6.19, p = 0.041) and of AFP (HR 1.02, 95% C.I. 1.001-1.03, p = 0.019) and DAA treatment failure (HR 5.68, 95% C.I. 1.63 to 19.80) (fig. 1) were significantly associated with higher risk of HCC recurrence, both at univariate and at Cox multivariable analysis.

Conclusion: Patients with lower risk of HCC recurrence are characterized by lower BMI, lower bilirubin and AFP levels and higher SVR rate. These data suggest that the absence of well-known HCC risk factors reduces the HCC recurrence rate also in patients underwent DAAs.

Figure:

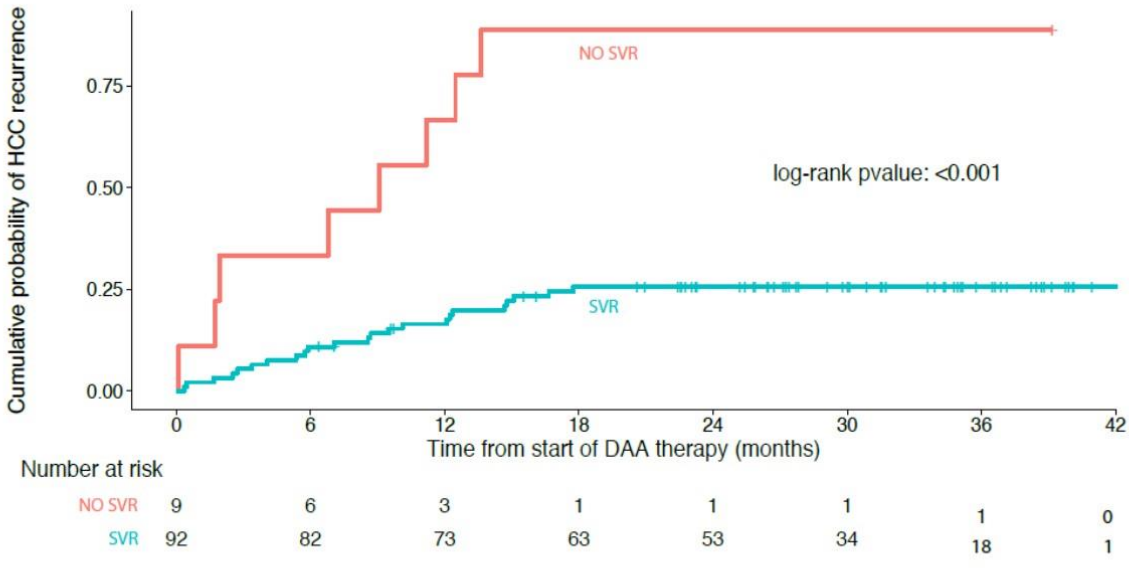

Figure 1. HCC recurrence risk according to SVR.

P06-03 Direct-acting antiviral treatment and recurrence of hepatocellular carcinoma: a single center experience

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Background and aims: A correlation between direct-acting antivirals (DAA) treatment and emergence of hepatocellular carcinoma (HCC) was reported. The aim of this study was to evaluate the rate of HCC recurrence after DAA treatment for HCV infection.
Method: From May 2015 to November 2018, we prospectively reviewed a series of patients with HCV cirrhosis and HCC under complete response after resection in 14% of patients, ablation in 63%, TACE in 10%, and combined treatment in 13%. Before starting DAA, CT scan was done to confirm the complete response. The rate of HCC recurrence was compared to that reported in the current literature in patients untreated with antivirals (6 months: 7.4%, 12 months: 20%, 24 months: 47%). The differences were analyzed by X2 test with a $p < 0.05$ considered significant.
Results: We consecutively enrolled 89 HCC patients (median age 72 years, 48-84) treated with DAA, 61% were males and 92% of patients had a well-preserved liver function (Child-Pugh A). SVR was observed in 95% of patients. The median follow-up was 23 months (range 1-42 months). Overall HCC recurrence was 35% and the median time of recurrence was 33 months (range 17-49). The cumulative probability of survival at 6, 12 and 24 months was 98.8%, 90.7% and 90.7%. The cumulative probability of HCC recurrence at 6, 12 and 24 months were 5.4%, 22.1% and 44% with no statistically significant difference compared to the outcomes reported in the current literature. Three months after DAA therapy, alpha-feto protein value decreased in 60% of patients as compared to basal value. BCLC stage at recurrence was 0 in 7% of cases, A in 59%, B in 24%, and C in 10%.
Conclusion: In our experience, DAA treatment is effective in achieving SVR in HCV-HCC patients. Short-term HCC recurrence is not increased and 2-year recurrence rate is also unaffected. The rate of HCC recurrence in our study was comparable to that reported in previous studies and positive effects of SVR on liver function might increase patient survival.

P06-04 The pattern of de novo hepatocellular carcinoma evolution in patients with hepatitis C virus following direct acting antiviral therapy needs to be clarified.

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The pattern of *de novo* hepatocellular carcinoma evolution in patients with hepatitis C virus following direct acting antivirals therapy needs to be clarified.

Background and aims: The evolution of de *novo* hepatocellular carcinoma (HCC) after direct acting antivirals (DAA) treatment is unknown. To describe the clinical evolution of patients with hepatitis C virus (HCV) treated with DAA who developed *de novo* HCC.
Method: We reviewed all HCV patients who were referred to the BCLC group from Feb/2012 to Dec/2017 due to HCC suspicion. Patients who have previously participated in clinical studies, which evaluated the *de novo* HCC incidence after of DAA treatment, were excluded. Variables from the baseline visit (first BCLC visit), when the HCC was confirmed and evolutionary events after HCC confirmation were recorded.
Results: We identified 480 patients HCV-HCC, of whom 92 were treated with DAA, 50 of them were excluded due to history of HCC and the remaining 42 were analyzed. The median age was 61.8 years and only 59.5% were male. At the time of HCC diagnosis, most patients were BCLC 0 (n = 13) or A (n = 22) and the remaining were B/C (n = 4/3), Child-Pugh-A (90.5%) and PS-0 (95.2%). During the follow-up 1 male patient developed spontaneous remission and in 4 (9.5%) the HCC progression precluded any treatment. In these 4 patients the median time from HCC diagnosis to the scheduled-day of the first HCC treatment (that could not be done) was 1.2 months. The treatment of the remaining 33 patients were surgery/hepatic transplant (n = 5), ablation (n = 20), loco-regional treatment (n = 7), systemic treatment (n = 1) and 4 were treated in other centers. Within 7.8 [IQR: 4.9-10.9] months after first HCC treatment 13/33patients (39.4%) treated in BCLC group developed HCC recurrence/progression. Nine of them received a second treatment [ablation (n = 5) and chemoembolization (n = 4) but the other 4 progressed to BCLC-D and received symptomatic treatment. Thus, after a median period of 7.6 [IQR: 6.1-14.5] months since HCC diagnosis, 8 patients either did not receive treatment due to progression to BCLC-D, or progressed to BCLC-D despite first HCC treatment (8/38;21.1%). Ten patients died, and tumor progression was the leading cause of death (50%).
Conclusion: The pattern of *de novo* hepatocellular carcinoma evolution in patients with HCV following DAA therapy varies from spontaneous remission to an aggressive course (21.1%). These data and the gender balance suggest that the mechanism involved in the HCC carcinogenesis in patients exposed to DAA requires an analysis of immunological and epigenetic factors.

P06-05 Evaluating the role of distinct innate and adaptive immune cells in causing liver cancer

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Background and aims: Chronic hepatitis in the liver is the main driver of liver cancer, the second most common cause for cancer related death in humans. Several different etiologies have been demonstrated to cause chronic liver disease with prominent inflammation including chronic viral infections with Hepatitis B or C (HBV, HCV) but also chronic alcohol consumption or chronic high caloric diet in combination with a sedentary life style.
Due to the consumption of high caloric food combined with increased sedentary lifestyle, overweight and obesity incidence has grown rapidly in Western countries (e.g. USA, Europe) but notably also in developing countries (*e.g*. India, China), affecting both adults and children. Although chronic viral infections are still the leading cause for hepatocellular carcinoma (HCC), alcoholic steatohepatitis (ASH), non-alcoholic fatty liver (NAFL) and subsequent non-alcoholic steatohepatitis (NASH) have become important etiologies for HCC.
Method: We and others have generated and characterized several pre-clinical mouse models that enable studying the mechanisms of inflammation induced liver cancer (e.g. NASH development and NASH to HCC transition in the context of a metabolic syndrome).
Results: Remarkably, these models recapitulated several human pathophysiological hallmarks of inflammation induced HCC. It has become apparent that adaptive immune cells but also innate immune cells play an important role in driving HCC-but at the same time actively participate in tumor surveillance.
Conclusion: Here, I will report on the different kinds of innate and adaptive immune cells that can drive primary liver cancer like NASH induced HCC or cholangiocarincoma (iCC) and will discuss the characterization and identification of novel targets to treat inflammation induced liver cancer (e.g. in the context of NASH).

P06-06 Somatic mutations and clonal dynamics in healthy and cirrhotic human liver

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Background and aims: Repeated liver injury predisposes to cirrhosis and hepatocellular carcinoma, but patterns of somatic mutation in chronic liver disease are unexplored.

Method: We sequenced whole genomes of 400 microdissections of 100-500 hepatocytes from 5 normal and 6 cirrhotic livers.

Results: Compared to normal liver, cirrhotic liver had higher mutation burden, especially structural variants, including chromothripsis. Cirrhotic nodules were oligoclonal; sometimes entirely derived from a single, recent common ancestor. Clonal expansions millimetres in diameter occurred in cirrhosis in the absence of known driver mutations. Endogenous mutational processes predominated, although signatures of exogenous mutagen exposure occurred in some samples. Up to 10-fold within-patient variation in activity of exogenous signatures existed between adjacent cirrhotic nodules, with both clone-specific and microenvironmental forces shaping this heterogeneity. Synchronous hepatocellular carcinomas exhibited the same repertoire of mutational signatures as background cirrhotic liver, but with higher burden.

Conclusion: Somatic mutations chronicle the exposures, toxicity, regeneration and clonal structure of liver tissue as it progresses from health to disease.

P06-07YI External beam radiotherapy (EBRT) as an effective and safe treatment in all stages of hepatocellular carcinoma (HCC) with cirrhotic liver diseHse: a single western institution experience.

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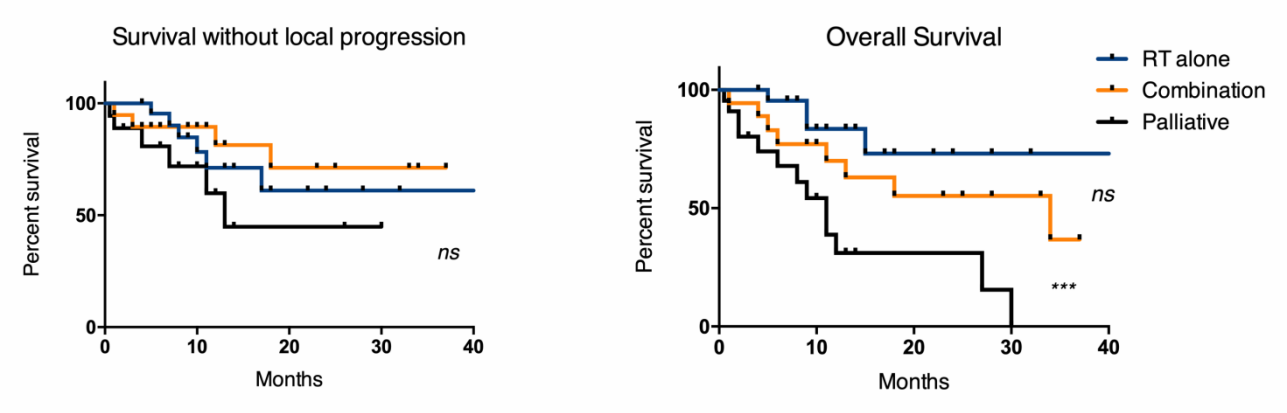
Background and aims: Recent data suggest that EBRT could be an efficient and safe local treatment of HCC. Yet, RT alone or in a combined strategy is rarely used for HCC patients.

Method: We retrospectively analysed all patients with any stage of HCC who received RT as a sole therapy or combined with other treatments. We reviewed charts of 66 patients treated between 01/11 and 09/18. Indications of RT were contraindication or technical issues for others local therapies, remaining active lesions after first local therapy or heavy comorbidities not allowing systemic treatments. We aimed to evaluate outcome and safety.

Results: Mean age was 70 ± 1 years, women 13 (20%), men 53 (80%). 61pts (92%) were cirrhotic among which 56% were Child A, B in 42% and C in 2%. The BCLC stage was A in 41%, B in 15%, C in 41% and D in 3% of pts. Median tumor size was 41 mm. 19 (29%) patients were naïve of treatment, 30 (64%) previously received one another HCC treatment and 17 (36%) received >1 previous treatment. RT was indicated as unique treatment, complementary treatment and as palliative therapy in 38%, 30%, and 32%, respectively. The median dose delivered was 42 Gray (Gy) with median of dose per fraction of 5 Gy, using 3D-conformal RT planning in 30% and stereotactic-body RT in 70% of pts. The median follow-up was 10.5 mths. 3 months after the end of RT, there were 17% complete responses (CR), 44% partial responses (PR), 35% tumor stability (TS) and 4% tumoral progression (TP). During overall follow-up, we observed 35% CR, 40% PR, 21% TS and only 4% TP. There was no statistical difference in terms of response between RT as unique treatment (21/23 patients) or combination treatment (13/20 patients) (p = 0.06). Among the 43 responders, response criteria were based on RECIST, mRECIST or both in 19%, 46% and 35% of cases, respectively. In the follow-up, 16 (24%) patients had no relapse, and among them, five patients underwent LT. 21% of patients had a local relapse after a median of 8.5 months, 53% had an intra-hepatic relapse at a median of 5.5 months and 19% had a metastatic relapse at a median of 4 months. 25 patients had subsequent treatment for a relapse. Tumor size was not statistically different in patients with or without local relapse (Mann-Whitney test, p = 0.07). RT was well tolerated; principal adverse events were asthenia (44% of patients), gastro-intestinal symptoms (14%), mild and transitory elevation of transaminases (9%) and signs of cirrhosis decompensating (9% of patients, requiring interruption of treatment). At the end of the study, 59% of patients were alive. Median overall survival was 34 months in the curative group and 11 months in the palliative group (Log-rank test, p = 0.0003).

Conclusion: RT should be considered as an effective and safe therapy alone or in combination in all stages of HCC and could be proposed for cirrhotic patients that present contraindication or failure for all others therapies.

Figure:


Survival without local progression
Percent survival
100
50
0
0
10
20
30
40
Months
ns
Overall Survival
RT alone
Combination
Palliative
Percent survival
100
50
0
0
10
20
30
40
Months
ns
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P06-08 AGT gene polymorphisms predict early dose modification of sorafenib for dermatological adverse events: towards tailored medicine for patients with hepatocellular carcinoma

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Background and aims: Sorafenib (SOR) is the standard of care of patients with advanced hepatocellular carcinoma (HCC). However, no biomarker exists able to stratify patients' response and predict treatment duration (TD) and overall survival (OS). It has been recently shown that the AGT gene polymorphisms (SNPs) can predict the early (<2 months) occurrence of treatment dermatological adverse events (eDAEs) requiring drug dose-reduction, which is associated with OS. We aimed to evaluate the ability of eDAE and genetic profile to predict the benefit of SOR in a large population of patients with HCC.
Method: We retrospectively evaluated 221 prospectively enrolled HCC patients treated with SOR (starting daily dose 800 mg) in five centers in Italy. Clinical and laboratory assessments were done monthly; interval of radiologic tumour re-evaluation was set according to single center's policy (2 or 3 months). Treatment was maintained until radiologic or symptomatic progression, significant toxicity or patient decision. Two SNPs (*rs699* and *rs4762*) of the AGT gene were assessed by using the TaqMan end point-genotyping assay. The end points of the study were the identification of baseline and time-dependant predictors of TD and OS and the correlation with ATG gene polymorphisms.
Results: Baseline characteristics of patients included in the analysis were: age 69 (26-86) years, 83% males, 46% HCV-pos, 86% Child-Pugh A, 61% Performance Status 0, 66% BCLC-C. Genetic distribution of AGT SNPs among the population were: 72 AA (33%), 101 AG (46%) and 47 GG (21%) for *rs699*; 172 GG (78%), 44 GA (20%) and 5 AA (2%) for *rs4762*. The median follow-up was 12 months (mos), the median TD 4 months and the OS at 24 months was 32% (CI 95% 26-39). At least one AE developed in 94% of patients; 46% patients required ≥1 dose reductions, which was due to AE in 88% and early in 62% of cases. eDAE developed in 26 patients (47% of those with an early reduction due to AE). Predictors of TD and OS by univariate analysis and time dependent covariate analysis are reported in Table 1 which shows that occurrence of eDAE was independently associated with longer TD and showed a trend with OS. The *rs4762* SNP was an independent predictor of eDAE (HR 3.01, 95% CI 1.14-7.99, p = 0.009), while the risk of eDAE was two-fold higher in carriers of at least one minor allele of both variants analysed (p = 0.031).
Conclusion: eDAE during SOR therapy predicted TD and OS and AGT *rs4762* SNP was associated with eDAE occurrence. Consequently, the AGT *rs4762* SNP could help to tailor treatment strategies in patients with HCC whenever different systemic therapies will become available.

P06-09YI Capecitabine in advanced hepatocellular carcinoma: a multicenter experience

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Background and aims: Sorafenib and Regorafenib are the standard of care in the treatment of advanced hepatocellular carcinoma (HCC). Aim of our study was to evaluate efficacy and safety of Capecitabine as an alternative in this subgroup of HCC patients.

Method: a cohort of 143 Capecitabine-treated patients was retrospectively analysed, which included 111 pts treated with Metronomic Scheme (MS, 1000 mg/day continuously) and 32 with Non-Metronomic Scheme (NMS, 2000 mg/day for 14 days followed by 7 days of interval).

Results: Patients were mostly BCLC C (130, 91%) with portal vein thrombosis (PVT) in 39% and metastatic disease in 52% of cases. NMS patients had significantly better Child-Pugh score, better ECOG-PS, smaller nodules and less frequently PVT (all p <0.05). Median treatment duration was 5.6 months, with an overall survival (OS) and a progression free survival (PFS) of 6.9 months [95% CI 5.73-8.13] and 2.6 months [95% CI 2.17-3.09], respectively. Survival was significantly longer for NMS than for MS (OS: 10.5 vs. 5.7 months, p = 0.0005; PFS: 3.9 vs. 2.5 months, p = 0.005). The disease control rate was 28.3% and the OS significantly correlated with radiological response (p = 0.0002). OS was 8.4 months [CI 95% 5.95-10.79] in patients intolerant to sorafenib versus 5 months [CI 95% 3.60-6.34] in patients with cancer progression (p = 0.024). The drug-related adverse events recorded were mainly haematological (thrombocytopenia, anemia, leukopenia), with no substantial differences between MS and NMS; 13 patients (9%) discontinued treatment because of side effects. Number of nodules, response to Capecitabine and additional therapy after Capecitabine were independent predictors of survival at Cox multivariate analysis.

Conclusion: Capecitabine confirms its safety and demonstrates clinical activity in patients with advanced HCC, particularly in patients intolerant to sorafenib and in those with mild impairment of liver function, without therapeutic alternatives. Conclusions cannot be drawn regarding the superiority of NMS over MS.

P06-10 Recurrence of hepatocellular carcinoma in patients with complete response treated with direct acting antivirals in clinical practice

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Background and aims: There is still controversy about the effect of Direct Acting Antivirals (DAA) on the early recurrence of Hepatocellular Carcinoma (HCC) in patients with complete response. The objective was to analyze the recurrence rate in patients with HCC who have received DAA and compare it with a control group who did not received antiviral treatment

Method: Retrospective, observational and multicentric study in 5 tertiary hospital in Madrid. Patients were selected from the Registry of Use of Antiviral Agents for HCV of Sermas (RUA-HCV) from November 2015-April 2016 all patients with HCC treated with surgery or ablation in complete response with at least 1-year of follow-up from the beginning of the DAA (Cohort A) and was compared with a consecutive historical group in the same centers that did not receive antiviral treatment (Cohort B). Clinical, radiological baseline and follow-up data were collected during DAA until the last visit

Results: We analyzed 665 patients treated with DAA registered in RUA-HCV. Finally, Cohort A, included 76 patients, mean age 66 years (SD9.2), males 57.9%, HIV co-infection 5.8%, diabetes mellitus 27.6%. BCLC 0: 28.9%, A: 65.8%, B: 5.3%. In control group, 57 patients with no antiviral treatment were included, mean age 65.3 (SD12.8), males 73.7%, HIV co-infection 16%, diabetes mellitus 31.6%. BCLC 0: 12.3%, A: 82.5%, B: 5.3%. The median follow-up from the beginning of DAA was 15 months and 25 months in control group. There were no statistically significant differences between recurrence at 3, 6, 12 and 18 months in Cohort A/B: 9.2%/3.5%, 15.8%/19.2%, 30.2%/29, 8%, 38.1%/38.5%, despite the fact that all patients in cohort A obtained a sustained viral response. In Cohort A, a higher recurrence was observed in patients treated in the first 12 months after treatment of HCC compared to those who were treated later (85.2% vs. 24.5%) p = <0.001. In fact, the cumulative probability of recurrence at 12, 18 and 24 months in patients who started DAA at a time ≤12 months compared with those who started >12 months was 25, 5% vs 0%, 42, 9% vs 3, 1% and 56, 3 vs 34% p = 0.001. A propensity score matching was made, not observing changes in the previously obtained results

Conclusion: There was no significant differences in terms of recurrence between patients treated with DAA and untreated. However, those patients treated at a time ≤12 months after complete response of HCC treatment showed a significantly higher recurrence than those treated at least 1 year after obtaining the complete response to the HCC.

P06-11YI Line1 retrotransposons-key drivers of hepatocellular carcinoma via TGF beta signalling

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Background and aims: Hepatocellular carcinoma (HCC) is the fourth most frequent cause of cancer-related deaths worldwide. The coexistence of chronic liver disease makes the use of traditional cytotoxic agents risky, with no survival benefit. Hence, there is an urgent need to develop novel treatment strategies. We have recently demonstrated activation and mutagenic consequences of long-interspersed repeat elements (L1) in HCC. Thus, we hypothesise that L1 reactivation plays a key role in hepatocarcinogenesis, which if defined may identify urgently needed novel therapeutic candidates for patients with HCC.
Method: The L1 expression was evaluated in patient biopsies by carrying out immunohistochemistry for L1-ORF1p. The staining was scored by a pathologist and associations with clinical characteristics were analysed using SPSS. Also, human HCC RNAseq dataset from TCGA-LIHC project was analysed for L1 transcript levels and correlation with clinical parameters and genetic mutations were determined. In parallel, L1 was knocked-down in HUH-7 using a shRNA construct. Cells were characterised by RNAseq to analyse changes in transcriptome and functionally by proliferation, colony formation and xenograft assays.
Results: All tumour (T) samples (n = 50) were positive for L1-staining but the intensity varied. None to low L1 staining was observed in non-tumour (NT) tissues. High L1 expression correlated with poorly differentiated tumour, high AFP levels and poor survival in TACE treated patients (mean survival 32.7 ± 8.4 and 15.2 ± 1.9 months for L1-low and high respectively, n = 25, p = 0.026). Likewise, L1 transcript level was found to be significantly upregulated in T compared to the matched NT. High L1 expression correlated with TP53 mutation, high AFP levels and activated TGFβ-signaling. There was no significant change in cell proliferation or colony forming ability upon L1 knockdown however, RNAseq analysis revealed several differentially expressed genes (DEGs) between Huh7_L1-KD cells and control cells. GSEA analysis of DEGs indicate dysregulation of several signalling pathways including TGFβ signalling. There was a significant delay in tumour development from Huh7_L1-KD cells compared to control in xenograft experiment in nude mice.
Conclusion: Higher L1 expression in tumours is associated with a subset of HCC patients with poorer prognosis and having TP53 mutation, high AFP and activated TGFβ signaling. *In vitro* experiments indicate a direct role of L1 in tumour biology and warrants exploration of L1 as a therapeutic target. Moreover, L1 can serve as a biomarker to guide use of TGFβ-inhibitors.

P06-12YI Survival and prognostic factors of patients with advanced hepatocellular carcinoma and treated with sorafinib

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Background and aims: Hepatocellular Carcinoma (HCC) carries a high incidence, prevalence and poor prognosis and survival particularly in developing countries where it may be diagnosed at an advanced stage. Sorafinib is the standard of care systemic treatment for patients with advanced HCC with Child-Pugh score A or carefully selected B. The aim of this work is to study the different prognostic factors that affected survival of advanced HCC patients treated with Sorafinib.
Method: this retrospective study included 69 Egyptian patients with advanced HCC who were treated with Sorafinib. They were recruited from the Multidisciplinary HCC clinic, Cairo University from January 2013 to June 2017. Demographic, laboratory and tumor characteristics were analyzed. Cox proportional hazard regression was used to detect the independent factors associated with survival.
Results: Patients were predominantly males and the median age (IQR) was 60 (54.5-65) years. All cases developed HCC on top of cirrhosis that was mainly due to HCV (83%). Most of our patients were Child-Pugh A (73%). The most common reported side effects are hand and foot disease, diarrhea and fatigue. The median overall survival was 12 months. Cox proportional hazard regression found that performance status of 2 or higher (p value 0.02, hazard ratio: 2.96 (1.22-7.21)) and size of focal lesion more than or equal 5cm (p value 0.03, HR: 2.36 (1.1-5.25)) were the independent prognostic factors of survival.
Conclusion: performance status of 2 or higher and size of focal lesion more than or equal 5cm were the independent prognostic factors of survival for advanced HCC Egyptian patients treated with Sorafinib.
Figure:
Characteristics of studied patients (69 patients)

Variable	Number (%)
median age (IQR)	60 (54.5-65)
Male gender	61 (88%)
Risk factors	
Smoker	32 (46%)
Parenteral anti-schistosoma treatment	37 (54%)
Family history	4 (6%)
Diabetes mellitus	17 (25%)
Hepatitis markers	
positive HBcAb total	1 (1%)
positive HBc IgM	0 (0%)
positive HBsAg	0 (0%)
HCV Ab	57 (83%)
Focal lesion characteristics	
prior ablation	8 (12%)
Portal vein thrombus	35 (50%)
Malignant lymphadenopathy	13 (19%)
Number of focal lesions	
Single	34 (49%)
Two	5 (7%)

Multiple	30 (44%)
Site of focal lesions	
Right lobe	43 (62%)
left lobe	4 (6%)
Bilobar	22 32%)
Performance status	
0	41 (59.4%)
1	22 (31.9%)
2	5 (7.2%)
3	1 (1.4%)
Child-Pugh	
A	50 (73%)
B	19 (27%)
Ascites	11 (16%)
ALT (n = 65)	
= <100	59 (85.5%)
>100	10 (15.5%)
AST (n = 65)	
= <100	47 (68.1%)
>100	22 (31.9%)
Total Bilirubin	
= <1.2	42 (61%)
>1.2	27 (39%)
AFP	
<8	8 (12%)
8-<200	19 (28%)
200-<400	6 (8%)
>400	36 (52%)

P06-13YI Predictive factors of tumor recurrence and overall survival in patients with hepatocellular carcinoma firstly treated by locoregional therapies: results of a retrospective cohort study.

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Background and aims: hepatocellular carcinoma (HCC) is the most common hepatic cancer worldwide, but predictive aspects of treatment response are still uncertain. We evaluated baseline predictive factors and their impact on disease course in HCC patient, treated with loco-regional therapies as first approach.
Methods: between 2011 and 2016, 168 HCC patients were enrolled at the Gastroenterology Unit of Molinette-University Hospital in Turin, Italy. Clinical, anthropometric and biochemical data were collected before treatment and during follow-up. We considered as primary end points tumor relapse and overall survival.
Results: in our cohort 132 patients were male; mean age was 64.8 ± 11. Liver disease etiology was HCV (83/168, 49, 4%), alcohol (28/168, 16, 7%), HBV (19/168, 11, 3%), NASH (8/168, 4, 8%) and multifactorial (HCV/HBV ± alcohol; 24/168). 140 (83%) patients had Child-Pugh score A, 27 (16%) B and 1 (0, 6%) C; 34 were BCLC 0, 114 A, 17 B and 3 (1, 8%) BCLC C or D (“bridge” therapy). At baseline, 54 patients had diabetes (32%), 76 patients presented metabolic syndrome features (53%), the mean waist circumference was 99 cm (± 9, 8), mean BMI was 26 (± 3, 5); biochemical results reported total cholesterol 142 ± 36 mg/dl, triglycerides 105 ± 56 mg/dl, albumin 3, 7 ± 0, 6, total bilirubin 1, 3 ± 0, 8 mg/dl, INR 1, 2 ± 0, 2; overall, 67 patients underwent antiviral therapy for either HBV or HCV infection. After a median follow-up of 40 months, HCC relapsed in 101 patients (60%). In our cohort no anthropometric, biochemical or baseline metabolic factors predicted tumor relapse or survival. At multivariate Cox-regression analysis, a baseline Child score ≥7 and viral active infection represented the two risk factors for recurrence (tumor relapse in high Child vs low Child: 57% vs 43%, p = 0, 005; viral active infection vs responders: 70% vs 30%, p = 0, 014). Furthermore, disease recurrence, baseline Child ≥7 and active viral infection were associated with lower survival (p = 0, 022 and 0, 004 respectively). Interestingly, NASH patients had a worse survival (Log-Rank 4, 04; p = 0, 044) than patients with other etiologies (5/8 NASH patients died, 4/5 for liver failure).
Conclusion: in HCC patients treated with loco-regional therapies as first approach, a baseline Child score ≥7 and viral active infection predict tumor relapse and, together with HCC recurrence and NASH-related liver disease, a worsened survival; no baseline anthropometric or metabolic factors predicted tumor relapse or survival.

P06-14YI Post-transplant de novo neoplasms: role of HCC in risk stratification

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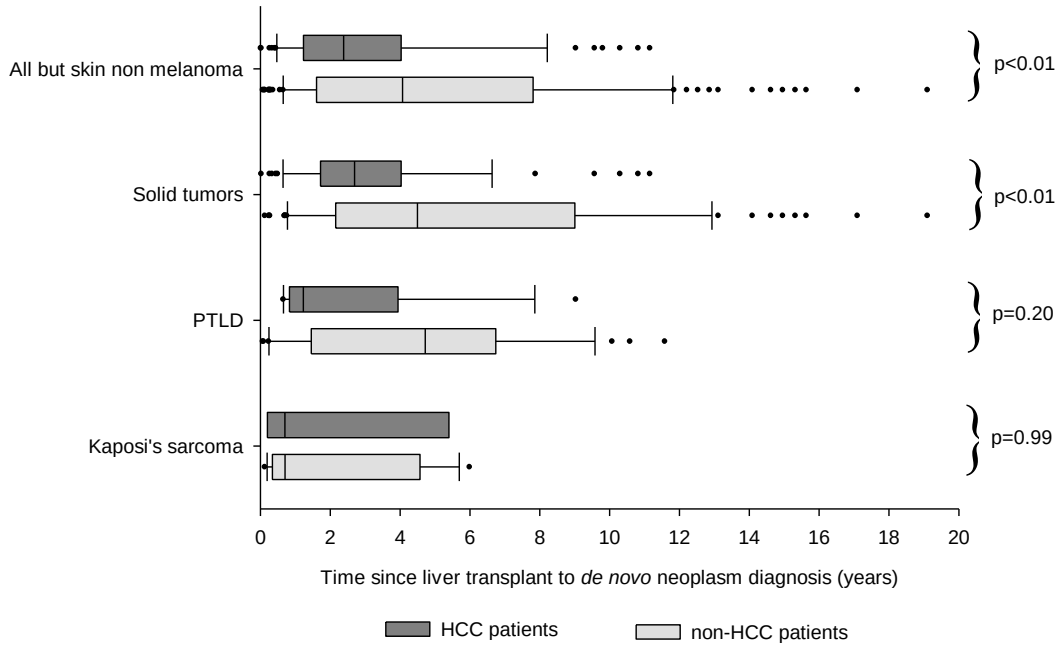
Background and aims: Patients with hepatocellular carcinoma (HCC) are at higher risk for second primary malignancies compared with general population. Such risk could be even higher after liver transplantation (LT). However, evidence on the additional risk that pre-LT HCC could confer to transplanted patients are lacking. As HCC has become the leading indication for LT, it is important to investigate whether such patients deserve more intensive post-LT screening for de novo neoplasms (DNN).

Method: A cohort study was conducted among 9 Italian centers between 1985-2014. Patients were excluded if: ≤18 years old, follow-up shorter than 90 days or cancer diagnosis within 90 days after LT. Person-years (PYs) at risk for DNN were computed from 90 days post-LT to date of death, cancer diagnosis or end of follow-up. Hazard ratios (HR) of DNN development and CI95% for patients transplanted for HCC (HCC patients) compared to those undergoing LT without any pre-transplant neoplastic history (non-HCC patients), were estimated. All models were adjusted for sex, age and calendar year at transplant, and liver disease etiology.

Results: A total of 2635 patients were followed up for 17, 903 PYs of observation [median: 5.6 years] during which 189 (7.1%) developed 202 DNNs. Out of 946 HCC-patients 62 (6.6%) developed 64 DNNs, while out of 1777 non-HCC patients 127 (7.4%) developed 138 DNNs. No significant association with the risk of all DNN emerged for HCC-patients as compared to non-HCC (HR = 1.33, 95%CI 0.94-1.86), after median follow-ups of 3.6 and 6.6 years, respectively ($p < 0.01$). In the analysis by specific tumor types, a significant increased risk emerged for bladder cancer only (HR = 12.75 95%CI 1.38-118.4). However, median time from LT to first DNN diagnosis was 2.4years for HCC-patients and 4.1years for non-HCC ($p < 0.01$). When differentiating by DNN macro-types this difference was relevant specifically for solid tumors (2.7 years vs 4.5 years, $p < 0.01$) (Figure 1).

Conclusion: In our cohort, HCC-transplanted patients were not at higher risk for DNN, except for bladder cancer, but presented earlier solid-tumors occurrence, probably due to their higher susceptibility to carcinogens and shared risk factors with primary cancer. Pre-trasplant liver neoplastic history could represent an additional risk factor for early solid-DNN occurrence, so that it should be taken into account during risk-stratification and surveillance-individualization.

Figure: Time since liver transplant to de novo neoplasm diagnosis



P07-01 Efficacy and safety of transarterial radioembolization with yttrium-90 in patients with hepatocellular carcinoma in the context of real clinical practice

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Background and aims: Transarterial Radioembolization (TARE) with Yttrium-90 is an emerging technique for the treatment of Hepatocellular Carcinoma with benefits not clearly established in terms of safety and efficacy. The objective was to evaluate the overall survival, adverse events and complications of TARE in clinical practice
Method: Observational, retrospective study of prospectively included patients treated with TARE from November 2011-July 2018 in clinical practice context. Clinical, analytical data related to the safety and efficacy of the technique were collected
Results: 45 patients were included. Male 80% (n = 36). Mean age 66 years (45-84). Diabetes mellitus 46.7% (n = 21). HCV 46.6% (n = 21), alcohol 28.9% (n = 13) and NAFLD 13.3% (n = 6). The Child-Pugh stage was A: 88.8% (n = 40) and B: 11.1% (n = 5). 51.1% (n = 23) had previously received ethanolization, ablation and/or chemoembolization. The BCLC stage was: A 15.6% (n = 7), B 68.9% (n = 31) and C 15.6% (n = 7). The ECOG-PS: 0 (66.7% n = 30) and 1 31.1% (n = 14). The mean albumin was 3.9 (DE0.4), bilirubin 1.2 (DE0.8), INR 1.0 (DE0.2) and MELD 9 (DE2.4). The median follow-up from TARE was 8.5 months (0.39-74.9). 22.2% have presented an episode of previous decompensation. The average dose of Yttrium-90 activity was 1.69Gbq. The complete response rate at 3, 6, 12, 18 and 24 months was 22%, 17.5%, 15%, 12.5% and 0% respectively. The partial response rate was 48.9%, 35%, 35% and 12.5% respectively. The average overall survival was 20.6 months. The overall survival at 3, 6 and 12 months was 88.9%, 75.9% and 58.9% respectively. There were no differences in survival between patients without progressing vs patients who progressed: 14.6 m [95% CI 0.4-28.8] vs 14.4 m [95% CI 10.2-18.7] (p = 0, 6). A 24.4% (n = 11) had early complications (<30d): ascites (36%), abdominal pain (27%) and asthenia (18%). The overall rate of post-TARE decompensation was 64.4% (n = 29), The most frequent was ascites. The post-TARE decompensation rate was 100% in patients with a previous episode of decompensation. The overall mortality rate was 57.7% (n = 26). The main factor related to mortality was decompensation (75.8% vs 25% p = <0.001)
Conclusion: TARE is an effective technique in tumor control in patients with hepatocellular carcinoma. Its clinical impact in terms of safety its affected due to the high rate of decompensation in advanced patients. The rigorous selection of patients with better liver function is necessary to achieve optimal results without harmful effects

P07-02 Thyroid hormone inhibits hepatocellular carcinoma development by reverting the metabolic reprogramming of cancer cells

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Background and aims: Altered energy metabolism of cancer cells, known as Warburg effect, has been widely recognized as one of hallmarks of cancer. The aim of this study was to investigate whether the observed anti-tumoral effect of thyroid hormone (T3) on hepatocellular carcinoma (HCC) development occurs via induction of a switch of the bioenergetic profile of cancer cells from glycolysis to oxidative phosphorylation (OXPHOS).
Method: The Resistant-Hepatocyte (R-H) rat model of hepatocarcinogenesis, which offers the possibility to identify distinct lesions (preneoplastic nodules, early and advanced HCCs) at well-defined timings, was used. A thorough metabolic analysis was performed by analyzing expression and protein levels, activity as well as modulation of several enzymes with key roles in glycolysis, pentose phosphate pathway (PPP) and OXPHOS.
Results: A one-week treatment with T3 reduced by 70% the number of preneoplastic lesions generated by the R-H protocol. Moreover, repeated treatment with T3 starting at a time when all rats already developed HCC (1 week every three weeks) significantly induced the regression of the tumors. The regression of pre- and neoplastic lesions induced by T3 was associated with a significant down-regulation of monocarboxylate transporter 4 (MCT4), glucose transporter 1 (GLUT1) and hexokinase 2 (HK2). T3 treatment also strongly inhibited both oxidative and non-oxidative branches of the PPP, as evaluated by the expression, protein and activity levels of glucose-6-phosphate dehydrogenase (G6PD), transaldolase (Taldo1) and transketolase (TKT). Furthermore, OXPHOS inhibition observed in pre- and neoplastic hepatic lesions of control animals, as demonstrated by the impaired activity of complex I and II of the respiratory chain, was rescued by T3 treatment. Regression of HCC was still evident even one month after T3 withdrawal.
Conclusion: Our results demonstrate that Warburg metabolic deregulation and PPP activation, which are early events in HCC development, can be reverted by T3. These data also suggest that the metabolic switch induced by T3 might be responsible for the powerful anti-tumorigenic effect exerted by this hormone.

P07-03YI Lack of CCL2 limits the development of HCC in a model of obesity-associated carcinogenesis

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Background and aims: Overweight and obesity are associated with increased cancer risk. Obesity-promoted HCC development is dependent on enhanced production of tumor- promoting cytokines IL-6 and TNF which cause hepatic inflammation and activation of the oncogenic transcription factor STAT3. CCL2 is a multifunctional chemokine involved in various aspects of the pathogenesis of chronic liver disease, including non-alcoholic steatohepatitis. Recently, the protective effects of the deficiency of CCR2, the receptor for CCL2, have been reported. However, the phenotype of ligand deficiency may be considerably different from that of chemokine receptors. In this study, we investigated the role of CCL2 deficiency in a model of HCC associated with fatty liver.
Method: 14 days old CCL2+/+ and CCL2 -/- mice were injected with DEN 25mg/kg IP or saline. After 4 weeks from the injection mice were fed with control (CD) or high-fat diet (HFD) for 38 weeks. Number and size of tumors were evaluated, Gene expression was measured by quantitative PCR.
Results: No differences in food consumption were observed across experimental groups. In CCL2 -/- mice body weight was 1.5 fold lower than in CCL2 +/+ mice. ALT levels were increased in all mice receiving HFD, whereas no effects of DEN were observed. The elevation was significantly higher in CCL2 +/+ mice. No tumors developed in animals not receiving DEN. In those exposed to the carcinogen, the number of tumors was significantly higher in mice on a HFD than in those on CD. The number and mean size of tumor was dramatically and significantly decreased in CCL2 -/- mice compared to wild-type. Gene expression analysis in the non-tumoral liver indicated that absence of CCL2 reduced the expression of TGF-beta and TNF-alpha. In the tumoral tissue, significantly reduced exoression levels of TNF-alpha, IL-18 and IL-6 were observed in CCL2-/- mice.
Conclusion: Genetic knock out of CCL2 is involved in tumor growth and progression in an obesity induced HCC murine model, and is associated with modulation of cytokine expression both in tumoral and non tumoral tissue

P07-04YI Circulating mir-4507 as a novel prognostic biomarker in hepatocellular carcinoma

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Background and aims: Despite the significant developments of therapeutic approach available for Hepatocellular Carcinoma (HCC), the efficacy remains poor due to the high recurrence and metastasis of HCC. Therefore, the identification of prognostic biomarker might be a crucial strategy to improve the survival of patient after therapy. This study aimed to investigate the potential of serum miR-4507 as a prognostic biomarker in patients with HCC.

Methods

A total of 100 HCC patients with HCC were enrolled and treated according to the EASL/AASLD practice guidelines. Serum samples were collected at the admission time. We conducted a discovery phase from twenty serum samples using miRNA 3.0 gene array (Affymetrix) microarray profiling analysis. miRNAs associated to therapy response, disease-free survival (DFS) and overall survival (OS) were selected for subsequent validation by using qRT-PCR. Relative quantification (fold of change) was determined using the $2^{(-\Delta\Delta Ct)}$ method. MiR-1280 was used as internal normalizer. NCSS statistical software was used to determine differences between groups in a Mann-Whitney U test procedure. The receiver operating characteristic (ROC) curves were plotted to estimate the prognostic value of the miRNA.

Results

Based on the miRNA selected from the discovery phase, the low miR-4507 expression was significantly associated with a poor prognosis after treatment. Serum miR-4507 was downregulated in patients with partial or no response after treatment ($p = 0.003$). The low miR-4507 expression was significantly associated to patients with DFS≤12 months ($p = 0.04$) and with OS≤12 months ($p = 0.022$). Circulating miR-4507 differentiates patients with a complete response to therapy from non-responders (AUC = 0.67, 0.54-0.76 95% CI) with a sensitivity and specificity of 69% and 67% respectively. The predicting value of the miRNA for longer DFS and OS showed an AUC of 0.64 (0.49-0.75 95%CI, 63% sensitivity, 60% specificity) and 0.62 (0.50-0.72 95% CI, 61% sensitivity, 55% specificity) respectively.

Conclusion

Serum miR-4507 provides a potential value as a novel biomarker for to predict prognosis after therapy in patients with HCC.

Funding

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P07-05YI Epidemiology, management and Barcelona clinic liver cancer staging status of Hepatocellular carcinoma: a tertiary care experience.

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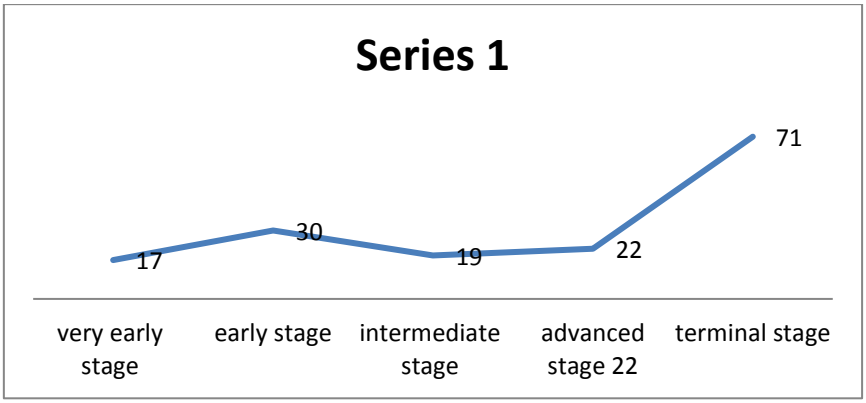
Background and aims: Hepatocellular carcinoma (HCC) is associated with a high degree of mortality and is one of the gravest complications of cirrhosis. In Pakistan its diagnosis is often made all too late; the disease is usually too advanced for therapeutic intervention.
To determine epidemiological factors, management and Barcelona Clinic Liver Cancer Staging (BCLC) status of the HCC at a tertiary care hospital.
Method: We undertook an observational, cross-sectional study at the Jinnah Postgraduate Medical Centre, Karachi during the period of January 2017 to October 2018. Male or female patients of age18≥years who were diagnosed as a case of HCC on CT-Scan Abdomen with contrast (Hepatoma protocol) were eligible for induction in this study. BCLC criterion was used to stage the disease and planning of intervention if any. Milan Criteria was used for candidacy of Liver transplantation.
Results: A total of 160 patients were inducted in the study. Majority of the patients were male 120 (75%). Ethnically Pathans 52 (32%) were the most predominant group. The mean duration for the diagnosis of HCC was just 1.45 ± 1.13 months with right upper quadrant pain 95 (59.4%) being the most common symptom. Chronic Hepatitis C infection was the most common risk factor in 94 (58.8%) of patients.
Most of the patients had multicentric 136 (85%) HCC and 88 (55%) also had portal vein thrombosis (PVT). A large number 71 (44.4%) had terminal stage disease according to BCLC staging; 113 (70.6%) patients were also not candidates for liver transplant according to Milan criteria.
Conclusion: Our study gives a dismal outcome with respect to HCC, even though most patients presented within months of diagnosis, the disease had already progressed to terminal stage. Most risk factors are preventable and need to be managed aggressively only then can there be any hope in managing and ultimately preventing HCC.
Figure:



P07-06 yttrium -90 trans arterial radioembolisation (tare) is an effective treatment for the downstaging of patients with hepatocellular carcinoma: a monocentric experience in a tertiary center

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Background and aims: Preliminary data in patients with unresectable hepatocellular carcinoma (HCC), suggest that Yttrium-90 trans-arterial radioembolisation (TARE) could be effective for downstaging of patients prior to liver transplantation or resection. The aim of this study is to evaluate the downstaging strategy with TARE in patients with unresectable HCC.

Method: A retrospective, observational study was performed using data collected from all consecutive patients undergoing TARE in a single tertiary center between October 2013 and June 2017. Patients could undergo TARE as the first line therapy or after progression to a previous treatment. Clinical, biological and imaging data were collected at baseline and during follow-up. Downstaging was considered achieved when a surgical treatment could be proposed after Recist and mRecist evaluation in morphologic follow-up imaging six months following TARE.

Results: 57 patients (Male = 88%, mean age 62 ± 10 years) were included: 48 (84%) of them have a compensated cirrhosis (Child Pugh class A and B in 46 (81%) and 11 (19%) patients, respectively). HCC was advanced according to Barcelona Clinic Liver Cancer (BCLC) in 58% of patients (n = 33). Most of the patients had an imaging infiltrative pattern (n = 32, 57%) and portal vein thrombosis (n = 33, 58%). In 58% of patients (n = 33), TARE was the first therapy. 42% of patients (n = 24) have achieved downstaging 6 months following TARE. Among these patients, 13 had undergone surgery: 9 (69%) were transplanted and 4 (31%) had liver resection. Median overall survival and progression free survival (PFS) of patients with or without downstaging was 389 days [284-739] and 197 days [126-333] (p <0, 001), and 271 days [166-571] and 98 days [60-178] (p <0, 001) respectively. In patients downstaged, PFS was higher in patients who underwent surgery (386 days [277-696]) compared to patients who didn't (199 days [150-236], p <0.001). The only variable independently associated to downstaging was diabetes (OR: 4.1[1.33-12.91]; p = 0, 014)

Conclusion: These preliminary results suggest that TARE is an effective treatment for downstaging of patients with unresectable HCC in almost 40% of patients, and allowed curative treatment in 54% of them. Combination of therapy with TARE followed by surgical treatment is associated with a 2 year-survival of 80% vs 0% in patients with no downstaging. However, these results need to be confirmed in a prospective study.

P07-07YI What are the predictive factors for long-term survivors with advanced hepatocellular carcinoma treated with sorafenib?

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Background and aims: Sorafenib is the standard therapy for advanced-stage hepatocellular carcinoma (HCC) and progressive disease after locoregional therapy. Most of these patients have an overall survival (OS) of about 10 months. However, some patients seem to have a better response to sorafenib and consequently a significantly prolonged survival time. Few data are available about this specific group. Our aim was to assess characteristics and predictive factors of long-term survivors (OS >24 months).
Method: We retrospectively reviewed 77 consecutive patients who started treatment with sorafenib for advanced-stage HCC (Barcelona Clinic Liver Cancer-C) or progressive HCC after locoregional therapy between Oct/2007 and Oct/2016.
Results: At time of initial sorafenib prescription, median age was 63.9 ± 10.4 years, mainly males (90.9%). All patients had cirrhosis and the most prevalent etiology was HCV infection in 41.6%, followed by alcohol-related disease (40.3%) and HBV infection (16.9%). The majority was Child-Pugh A (66.2%), had multinodular disease (74%) and had alpha-fetoprotein (AFP) level <400ng/ml (63.6%). Portal vein thrombosis (PVT) was present in 50.6%. BCLC distribution: 96.1% in stage-C and 3.9% in D. Time between HCC diagnosis and start of sorafenib was 7.9 ± 9.8 months. In most cases, sorafenib was the first-line treatment (62.3%). For those with previous treatment, transarterial chemoembolization was the most performed technique (58.6%).
Mean OS was 32.6 months (95% CI: 23.4-41.8) with 2-year survival rate of 38.9%. Fifty-four patients died (70.1%). Twenty-five patients had a survival time superior to 24 months (32.5%) after initiating sorafenib. In this subgroup, mean age was 60 ± 7.7 years. Most patients were male (80%) and had a HCV infection-related disease (52%). Ninety-two percent were Child-Pugh A (mean value: 5.6 ± 0.8 points), AFP level was <400ng/ml in 84% and mean MELD-Na^+ score was 10.1 ± 2.7 points. The majority presented with multinodular disease (64%) with absence of PVT (80%).
In multivariate analysis, predictive factors significantly related to long-term survival were: absence of portal vein thrombosis (p = 0.004, OR 0.123, 95% CI: 0.031-0.491) and MELD-Na^+ score (p = 0.037, OR 0.870, 95% CI: 0.764-0.992).
Conclusion: Although mean OS in this group of patients is low, it is of note that a sub-group can really benefit from this treatment and it is worth identifying those long-term survivors, in our study those without PVT and a lower MELD-Na^+.

P07-08 Efficacy and safety of the combination of pravastatin and sorafenib as a new therapy for the treatment of advanced hepatocellular carcinoma

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Background and aims: Statins regulate the cholesterol biosynthesis and have antineoplastic properties. The present study analyzes the efficacy and safety of the combination of sorafenib + pravastatin measured by overall survival (OS) and progression-free period in patients with advanced hepatocellular carcinoma (HCC).
Method: A phase II, multicenter, randomized, double-blind, placebo-controlled clinical trial was conducted, in which patients with advanced HCC candidates for systemic treatment with sorafenib (BCLC stage B or C) were selected, with ECOG performance status 0-2 and preserved liver function (Child-Pugh A or B7). They were randomized in a stratified manner by center and by risk factors (vascular invasion and extrahepatic metastases) in a 1:1 ratio to receive placebo + sorafenib (control group) vs. pravastatin + sorafenib (experimental group). The maximum duration of treatment was 18 months. Physical examinations, clinical and analytical evaluations, and radiological tests were performed every 8 weeks from the baseline visit. The treatment was maintained until progression, death or unacceptable toxicity. The main objectives were to evaluate OS and time to disease progression (TTP).
Results: A total of 35 patients were selected, of which 31 patients were randomized (placebo + sorafenib = 16, pravastatin + sorafenib = 15). Clinical characteristics were the same in both arms: mean age = 61 years, 93% males, 90% Child A, and 77% BCLC C. Median time of treatment was 9.5 months for pravastatin + sorafenib and 3.4 months for placebo + sorafenib. The median OS was slightly higher in the experimental group but not significant (12.4 months vs. 11.6 months in control). The radiological TTP was significantly higher in pravastatin + sorafenib (9.9 months vs. 3.2 months, p = 0.008) than in the control group. Serious adverse events (SAE) accounted for 10.4% of the total adverse events (AE) (36.8% of them in the experimental group and 63.2% in the control group), none of them related to the drug in study. The definitive interruption by AE was 3.2% in the pravastatin + sorafenib group and 9.7% in the placebo + sorafenib group.
Conclusion: The combination of pravastatin + sorafenib prolonged the TTP of patients with advanced HCC, but did not improve the OS compared to sorafenib alone. The combination treatment was safe and well tolerated, and suggests certain therapeutic value that should be confirm and explore further.

P07-09YI miR-21/PPARalpha activation in NASH promotes progression towards hepatocellular carcinoma

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Background and aims: The molecular mechanisms governing the progression of non-alcoholic steatohepatitis (NASH) towards hepatocellular carcinoma (HCC) remains elusive. We have recently shown that concomitant miRNA-21 (miR-21) ablation and farnesoid X receptor (FXR) activation prevents NASH development in mice. Here, we aimed to evaluate the role of the miR-21/PPARα pathway in NASH-prompted carcinogenesis.

Method: Wild-type (WT) and miR-21 KO C57BL/6N 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. Serum was collected for biochemical analyses and liver samples processed for histological analysis and measurement of miR-21, PPARα and metabolic relevant genes, as well as pro-inflammatory/pro-fibrogenic cytokines. A profiler PCR array was used to evaluate the expression of liver cancer-related genes.

Results: WT mice fed the CDAA diet for 32 weeks developed macrovesicular steatosis, hepatocyte ballooning, NASH and fibrosis, concomitantly with accumulation of perivascular lymphoid cells and macrophage agglomerates. CDAA-fed miR-21 KO mice exhibited increased activation of PPARα target genes, augmented mitochondrial activity and decreased fatty acid serum levels, compared with WT mice. After 66 weeks, all WT mice on the CDAA diet 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 and its direct transcriptional genes. In addition, livers presented with mitochondrial dysfunction, hyperplastic foci and anisokaryosis, as well as phenotypically altered and highly proliferative (Ki-67 positive) hepatocytes. Increased levels of pro-inflammatory/fibrogenic markers were particularlly evident in pre-neoplastic liver tissues, alongside higher activation of oncogenic pathways. Strikingly, CDAA-fed miR-21 KO mice for 66 weeks displayed serum ALT levels similar to control animals and, compared with CDAA WT-fed mice, the NAS score (<5); number of liver nodules (~2.3 nodules/animal); hepatocyte profliferation and expression of oncogenes were all significantly reduced, and the pro-inflammatory/fibrogenic milleau reversed to almost baseline controls.

Conclusion: Overall, activation of the miR-21/PPARα pathway appears to contribute to NASH-associated carcinogenesis, with its inhibiton halting HCC development. Targeting miR-21 and/or PPARα may constitute an appealing therapeutic approach to ameilorate NASH and its progression towards HCC. (PTDC/BIM-MEC/0895/2014, SFRH/BD/88212/2012, SAICTPAC/0019/2015 FCT, PT).

P07-10 Integrative analysis defines distinct prognostic subgroups of intrahepatic cholangiocarcinoma

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Background and aims: Intrahepatic cholangiocarcinoma (iCCA) is the second most common primary liver cancer. It is defined by cholangiocytic differentiation and has poor prognosis. Recently, epigenetic processes have been shown to play an important role in cholangiocarcinogenesis.

Method: We performed an integrative analysis on 52 iCCAs using both genetic and epigenetic data with specific focus on DNA methylation components. Therefore, we performed DNA methylation profiling and panel sequencing. In addition, copy number alterations were assessed based on the signal intensities measured in the methylation array. MeDeCom analysis was performed to dissect methylation patterns into latent methylation components (LMCs) to incorporate information on possible cell type composition differences.

Results: We found recurrent IDH1 and IDH2 (28%) gene mutations, recurrent arm-length copy number alterations (CNAs), and focal alterations such as deletion of 3p21 or amplification of 12q15 which affect BAP1, PBRM1, and MDM2. DNA methylome analysis revealed excessive hypermethylation of iCCA, mainly affecting bivalent genomic regions marked with both active and repressive histone modifications. Integrative clustering of genetic and epigenetic data identified four iCCA subgroups with prognostic relevance further designated as IDH, high (H), medium (M) and low (L) alterations group. The IDH group comprised all samples with IDH1 or IDH2 mutations and showed, together with the H group, a highly disrupted genome, characterized by frequent deletions of chromosome arms 3p and 6q. Both groups showed excessive hypermethylation with distinct patterns. The M group showed intermediate characteristics regarding both genetic and epigenetic marks, whereas the L group exhibited few methylation changes and mutations and a lack of CNAs. Methylation-based latent component analysis of cell-type composition identified differences between these four groups. Prognosis of the H and M groups was significantly worse than that of the L group.

Conclusion: Using an integrative genomic and epigenomic analysis approach, we identified four major iCCA subgroups with widespread genomic and epigenomic differences and prognostic implications. Furthermore, our data suggest differences in the cell-of-origin of the iCCA subtypes.

P07-11YI Obeticholic acid decreased alpha-Feto-protein levels in HCC through Farnesoid X receptor (FXR)

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Background and aims: Farnesoid X receptor (FXR) highly expressed in the liver and intestines and regulates the expression of a wide variety of genes implicated in the metabolism of bile acids, glucose and lipids. Recently, there is increasing evidence suggesting that activation of FXR may have beneficial functions in inhibiting liver fibrosis and cirrhosis. We aimed to explore modulatory effects of FXR agonists on Hep3B cell line.
Method: Hep3B cells (human hepatocellular carcinoma) were treated with 10mM and 50mM of Obeticholic acid (OCA). Following 24 h, HCC were washed and counted for evaluating apoptosis/necrosis (Annexin-V/PI) by the flow cytometry. Medium were collected for α-Feto-protein (αFP) measurements by ELISA. Western blot and confocal microscopy were used to evaluate expressions of FXR.
Results: OCA-treated HCC activated protein FXR expressions. In parallel, OCA while decreased apoptosis of HCC from 60 ± 7% to 39 ± 3.1% (annexin-V+/PI-) it shifted the cells to late apoptosis and necrosis from 26 ± 4 to 50 ± 6.6 % (annexin-V+/PI+). αFP levels were reduced from 250 ± 27 ng/ml in untreated HCC to 89 ± 11 ng/ml in the OCA-treated cells ($p < 0.05$). There results were exacerbated with 50mM OCA.
Conclusion: Our results suggest anti-cancer effects of OCA on HCC is mediated by FXR modulations. Modulatory therapies targeting FXR could be a potential to prevent complications to liver cirrhosis.

P07-12YI Incidence of hepatocellular carcinoma after hepatitis C cure with DAA in a cohort of patients with advanced liver disease. Results from a prospective screening program

Marco Sanduzzi Zamparelli[1], Sabela Lens[2], Víctor Sapena[1], Neus Llarch[1], Anna Pla[2], Gemma Iserte[1], Concepció Bartres[2], Ramón Vilana[3], Luis Bianchi[3], Nuñez Isabel[4], Anna Darnell[3], Ernest Belmonte[3], Carmen Ayuso[3], Maria Ángeles García-Criado[3], Alba Díaz[5], Carla Fuster[5], Ferran Torres[6 7], Jordi Bruix[1], Xavier Forns[2], María Reig[1], Zoe Mariño[2]

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Background and aims: Although HCV cure with DAA (>95%) is associated with a reduced risk of disease progression, the impact of DAA on hepatocellular carcinoma (HCC) occurrence is controversial. The aim of this study was to estimate HCC incidence after DAA in a cohort of patients with advanced liver disease by means of a prospective screening programme.

Method: Prospective study including HCV-infected patients with cirrhosis or advanced fibrosis (F3, TE≥9.5Kpa), without previous history of HCC, cured after DAA; patients should have a US imaging in <30 days from inclusion excluding the presence of HCC or non-characterized nodules. All patients were evaluated every 6 months. Follow-up (FU) time was censored at the moment of event (HCC) or September 2018. HCC incidence was expressed in 100/patients-year (100PY) (IC95%). Adherence to screening was assessed.

Results: 275 patients signed inform consent; 90 patients were excluded (mainly due the absence of pre-DAA US in <30 days); 185 patients were analysed: 52.4% men, age 65.1 [55.1-72]years. 34% (n = 63) patients were F3 (TE 11.5[10.1-12.1]KPa) vs 122 (65.9%) patients with cirrhosis (TE 18[14.3-26.6]Kpa): 87.7% Child-A, 17.2% history of decompensation, 40.9% varices in endoscopy, 39.3% TE≥21Kpa. Adherence to screening program was 98.4% and 7 incident HCC were detected after a median clinical and radiological time of 27.5[24.7-33.9] and 23.9[23.4-24] months, respectively. Median time from SVR to HCC diagnosis was 24.5[17.3-30.7] months. Overall incidence of HCC was 2.01/100PY[IC95%: 0.9-4.2]. All HCC cases occurred in cirrhotic patients (incidence: 3.04/100PY[IC95%: 1.4-6.3]) with TE≥21KPa (incidence in subgroup: 5, 93/100PY[IC95%: 2.9-11.8]. The 7 HCC cases [BCLC-0 (n = 3)/A (n = 3)/C (n = 1)] received specific treatment [percutaneous (n = 4), surgery (n = 1), TACE (n = 1); sorafenib (n = 1)]; 2 patients presented recurrence/HCC progression after 2.3 and 2.04 months of oncologic treatment. During FU, 7 patients died (3.78%), one due to HCC progression.

Conclusion: Risk of HCC persists in cirrhosis even if SVR is achieved with DAA (3.04/100PY). In this cohort, we did not identify any HCC in F3 patients (although the number of patients is limited). Moreover, in this specific cohort of patient without non-characterized nodules at baseline we did not find a time-association of HCC and DAA. Altogether, screening programs to rule out HCC are necessary in patients with cirrhosis achieving SVR; this risk should be further investigated in larger cohorts of F3 patients.

P07-13YI Sorafenib for patients with recurrent hepatocellular carcinoma after liver transplantation: intrinsic resistance or not?

Francesco Tovoli[1], Giovan Giuseppe Di Costanzo[2], Vito Sansone[1], Giulia Magini[3], Rodolfo Sacco[4], Tiziana Pressiani[5], Franco Trevisani[1], Raffaella Tortora[2], Laura Bucci[1], Maria Grazia Lucà[3], Luigi Bolondi[1]

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Background and aims: the use of sorafenib in patients with hepatocellular carcinoma (HCC) recurring after orthotopic liver transplantation (OLT) may be challenging. Increased toxicity from interaction with immunosuppressants are a common issue. Also, poor outcomes have been reported. However, it has not been investigated whether these outcomes derive from a dire clinical presentation or from a resistance to sorafenib. We aimed to compare the overall survival (OS) of sorafenib-treated post-OLT HCC with that other sorafenib-treated HCCs.

Method: We analyzed a large retrospective-prospective database gathering the clinical data of 487 patients from 6 Italian centres, who were prescribed with sorafenib between 2008 and 2017. Eighteen patients with HCC recurring after OLT were identified. A propensity score analysis comparing their clinical and tumor characteristics with those of 90 matched controls (sorafenib in patients without OLT) was performed. Propensity score included performance status, alfa-fetoprotein >400 ng/ml, macrovascular invasion, extrahepatic spread.

Results: Characteristics of the OLT patients and matched controls are reported in the Table. Immunosuppressant in OLT patients included: everolimus (n = 10), tacrolimus (n = 4), sirolimus (n = 2), cyclosporine (n = 2). Toxicities were similar in the two groups.The median treatment duration [4.5 months (95%CI 3.0-6.1) vs 5.9 months (95% CI 4.0-7.9), p = 0.344] was comparable as well as the rate of radiologic disease control (44.4 vs 52.5%, p = 0.323). Finally, the OS was also similar [11.5 months (95%CI 9.2-13.8) vs 13.5 months (95%CI 9.7-17.3), p = 0.725] in OLT and non-OLT groups, respectively.

Conclusion: Extrahepatic spread was common in patients with HCC recurring after OLT, possibly reflecting the negative effects of immunomodulation.. However, once sorafenib was started, treatment duration, radiological response and OS were comparable with those of controls. The prognosis of these patients seems to be more influenced by their clinical presentation rather than by a reduced efficacy of sorafenib.

Figure:

	OLT patients (n = 18)	***Sorafenib controls (n = 90)***	*P*
Males	16 (88.9%)	80 (88.9%)	1.000
Age	59 (56-67)	65 (62-74)	0.101
Performance status			
-ECOG-PS 0	14 (77.8%)	71 (78.9%)	1.000
-ECOG-PS 1	4 (22.2%)	19 (21.1%)	
BCLC stage			
-Intermediate	4 (22.2%)	21 (22.2%)	1.000
-Advanced	14 (77.8%)	69 (73.8%)	
Macrovascular invasion	2 (11.1%)	16 (17.8%)	1.000
Extrahepatic spread	13 (72.2%)	65 (72.2%)	1.000
Alfa-fetoprotein >400 ng/ml	4 (22.2%)	19 (21.1%)	1.000

P07-14 Sequential systemic treatment in advanced hepatocellular carcinoma prolongs median overall survival to more than three years.

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Background and aims: The number of efficacious systemic agents for advanced hepatocellular carcinoma (HCC) has rapidly increased over the past two years. However, guidance for optimal sequential systemic treatment in patients with advanced disease and experience with outcome and safety profile are lacking. Thus, we aimed to assess efficacy and tolerability of sequential systemic therapy of HCC.
Method: Our single-center study prospectively followed 14 patients who received multiple, sequential systemic therapies after progression or intolerance to sorafenib. End points were overall and progression-free survival (OS, PFS), objective response rate (ORR), and treatment emergent adverse events (TEAE).
Results: Patients had well compensated liver function and good performance status at start of each systemic therapy. Agents included sorafenib (n = 14), regorafenib (n = 10), immunotherapy with nivolumab or pembrolizumab (n = 10), lenvatinib (n = 3), ramucirumab (n = 2), and others, with a median of three lines of systemic therapy per patient. Median OS was 37.4 months from initiation of 1st line therapy with sorafenib. PFS and ORR for sorafenib, regorafenib (TKI), and immunotherapy were 6.6, 5.3, and 6.6 months, and 15.4%, 11.1%, and 22.2%, respectively. TEAE were frequent (46-80%), but mostly manageable during TKI therapy without the need for termination. However, TEAEs due to immunotherapy (60%) led to cessation of treatment in 40% of patients.
Conclusion: Sequential systemic therapy prolongs median OS in selected patients with advanced HCC to more than three years. TEAE are frequent, but manageable, and the quality of adverse events depends on the respective agent. Further investigation of potential predictive biomarkers is needed.

P07-15YI Dual role of C3G in hepatocarcinoma tumor growth and progression. Implication in the HGF/c-Met signaling pathway

CELIA SEQUERA HURTADO[1], Sara Manzano[1], María Arechederra[1], Paloma Bragado[2], Cristina Baquero[1], Nerea Palao[1], Luis Hernández-Cano[3], Carmen Guerrero[3], Almudena Porras[1]
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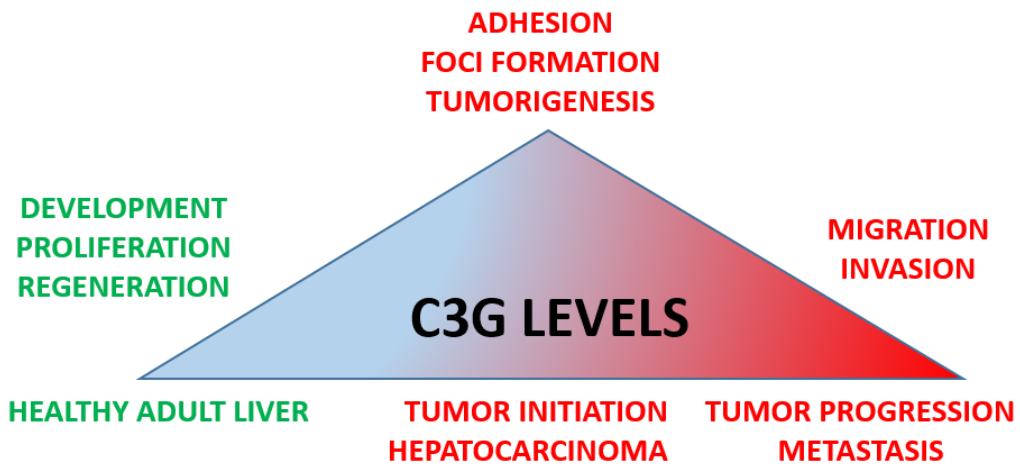
Background and aims: C3G (RapGEF1) is a guanine nucleotide exchange factor for Rap1 and R-Ras. It is essential for embryonic development and regulates several cellular functions such as cytoskeletal remodeling, differentiation and cell death. However, its role in cancer is controversial acting either as a tumor promoter or suppressor. Using different public genomic data bases, we found that C3G expression increases in tumor samples from hepatocarcinoma (HCC) patients as compared to control livers, while it is reduced in metastatic samples. Accordingly, a Kaplan-Meir analysis revealed a shorter survival in patients bearing mutated C3G versus control patients. In agreement with this, we found that C3G protein levels are higher in HCC cells than in adult hepatocytes, as well as in mouse liver tumors generated by c-Met overexpression. Additionally, differences in the expression of C3G isoforms was detected. Furthermore, our group found that C3G knock-down increased invasion and migration of hepatocarcinoma (HCC) cells, partially due to the induction of an epithelial-mesenchymal transition (EMT) process. Based on the information obtained in the genomic human databases and on the pro-invasive effect of C3G knock-down in HCC cells, we wanted to characterize the role of C3G in HCC growth and progression.
Method: We evaluated the effect of permanent C3G knock-down on *in vitro* (anchorage-dependent and independent) and *in vivo* (xenografts, metastasis assay) growth of different HCC cells with a more epithelial or mesenchymal phenotype (Hep3B and HLE). Its effect on adhesion, cell death and stemness capacity was also studied. The signaling pathways involved in these biological processes was also analyzed by western-blot.
Results: We found that C3G promotes in vitro tumor growth by enhancing anchorage-dependent growth. In contrast, C3G inhibits migration and invasion. Moreover, in vivo tumor growth in nude mice was reduced upon C3G knock-down in Hep3B, while the dissemination of cancer cells to bone marrow increased, indicating a greater metastatic capacity. In contrast, the fibroblast infiltration in the tumor stroma was lower in this case based on alfa-SMA staining. Moreover, according to the increased expression of C3G in mouse liver tumors overexpressing c-Met, we found that activation of HGF/c-Met pathway was defective in HCC cells subjected to C3G downregulation.
Conclusion: Our data support a dual function of C3G in HCC. On one side, C3G acts as a tumour promoter by enhancing adhesion, anchorage-dependent growth and in vivo tumour growth. On the other hand, C3G inhibits migration, invasion and anchorage independent growth, reduces stemness and increases cell death of HCC cells. Furthermore, our data suggest that C3G is required for the full activation of c-Met and its downstream pathways in this context.
Figure:



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Industry interactive sessions

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Friday 15 February 2019, 12:30-13:15 – Room: Auditorium VIII

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Leveraging sequential systemic therapy to prolong patient survival
Chairs Jordi Bruix, *Spain*
Peter Galle, *Germany*

12:30 - 12:35	Opening remarks Peter Galle
12:35 - 12:50	Standardized approach to using first and subsequent lines of therapy Jordi Bruix
12:50 - 13:05	Strategies to optimize systemic treatments to prolong OS Peter Galle
13:05 - 13:15	Discussion, debate, and questions and answers Panelists: Jordi Bruix, Peter Galle
13:15	Closing remarks Jordi Bruix

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Thursday 14 February 2019, 20:00-20:45 – Room: Auditorium VIII

Cancer immunotherapy in HCC: Implications for a multi-disciplinary approach
Chairs: Lorenza Rimassa, *Italy*
Arndt Vogel, *Germany*

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Friday 15 February 2019, 19:20-20:05 – Room: Auditorium VIII

Treating patients with HCC: When, how, why?
Chairs: Thomas Decaens, *France*
Marcus-Alexander Wörns, *Germany*

19:20 - 19:25	Welcome and introductions Thomas Decaens
19:25 - 19:40	Clinical case 1 From TACE to systemic therapy: not too early, but not too late Thomas Decaens
19:40 - 19:55	Clinical case 2 From 1st to 2nd line systemic therapy: the wider the choice, the harder to choose Marcus-Alexander Wörns
19:55 - 20:05	Discussion and close Thomas Decaens

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February 2019

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Auditorium VIII, Congress Center Lisbon

FACULTY

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Professor Marcus-Alexander Wörns (Germany)

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Treating patients with HCC

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Innovation for patient care

CBZ-PT-000031
Date of preparation: January 2019