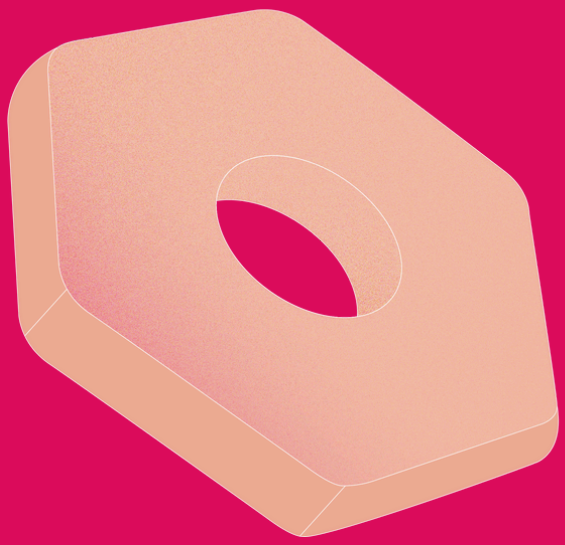
EASL LIVER CANCER SUMMIT 2025

Paris, France
20-22 February



ABSTRACT BOOK

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ORAL ABSTRACT PRESENTATIONS

OS-1
Intratumor heterogeneity is conserved in single cell-derived hepatocellular carcinoma organoids

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Background and aims: Hepatocellular carcinoma (HCC) is characterized by significant intratumoral heterogeneity, a feature associated with therapy resistance and recurrence. HCC organoids (HCCOs) are a valuable tool to study tumor biology, as they have been described to recapitulate the intratumoral heterogeneity of the originating tumor. This study aims to investigate the mechanisms by which intratumoral heterogeneity is maintained within HCCOs over time and to evaluate how this diversity impacts therapeutic efficacy.

Method: To assess whether intratumoral heterogeneity is preserved in single cells (sc), we isolated individual cells from HCCO cultures, which inherently exhibit heterogeneous tumor marker expression. These single cells were plated individually in ultralow-attachment plates, allowing them to regenerate into organoids. The resulting single-cell-derived organoids (sc-HCCOs) were analyzed by immunohistochemistry (IHC) to evaluate the expression of liver cancer-related markers that were variably expressed in both the original HCCOs and the corresponding tumor biopsy. Additionally, we assessed the proliferation rates of sc-HCCOs and their response to Sorafenib treatment to determine the impact of heterogeneity on drug sensitivity.

Results: sc-HCCOs reproduced the heterogeneity of tumor marker expression observed in the original HCCO lines, as demonstrated by immunohistochemical analysis. sc-HCCOs exhibited variability in proliferation rates and response to Sorafenib, but this variability was small compared to the differences observed between the original HCCO lines.

Conclusion: Our findings demonstrate that sc-HCCOs successfully reproduce the heterogeneity of tumor marker expression observed in the original organoids. While we observed some variability in proliferation rates and response to Sorafenib among the sc-HCCOs, the extent of this variability was limited. These results suggest that while single-cell-derived organoids retain certain aspects of tumor heterogeneity, the implications for functional diversity in terms of proliferation and drug response remain unclear. Further investigation is needed to better understand the underlying mechanisms and their relevance to the intratumoral heterogeneity of HCC.

OS-2
The tumor suppressor miR-122 regulates liver cancer stem cells immune evasion and proliferation through CD24

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Background and aims: We have previously shown that liver cancer stem cells are a source of primary liver cancer. New factors, in particular immunological, are essential for converting liver cancer cells to immune targets. CD24 characterizes tumor stem cell and is a driver of liver cancer and as a “don’t-eat-me-signal”, repelling an immune affect against the cells. However, it is not known what increases CD24 expression and how this increase affects hepatic tissue and enhances hepatocarcinogenesis.

Method: To unfold the mechanism of how hepatic cancer stem cells proliferation is regulated and escapes the anti-cancer immune response, we performed *in vitro* and *in vivo* experiments, including therapeutics *in vivo* and performed analysis of human liver cancer samples and samples from chronic disease patients.

Results: We found that in chronic liver inflammation, both autoimmune and MASH, ductular reaction (DR) develops and is dependent on CD24 expression and signalling. We show that liver tumor cell propagation is dependent on CD24 expression and that CD24 expression is increased in livers of both human and mouse pre-malignant conditions and CCA and mixed HCC-CCA samples. To determine how CD24 is regulated, we found that microRNA miR-122, also known to act as a tumor suppressor, regulates CD24 by repressing its expression by targeting the miR seed in its 3’-UTR. We found that increasing miR-122 expression with compounds that we developed, that act to increase the activity of RORà, a known activator of the miR-122 promoter, leads to suppression of CD24 and DR. In addition, miR-122 regulates the do-not-eat-me signal of CD24. Furthermore, treating Mdr2-KO mice with anti-CD24, exhibited a therapeutic effect against DR and liver fibrosis. In addition, we investigated what causes miR-122 levels to decrease and enable CD24 expression and the development of DR. We found that upon liver inflammation, pSTAT3 activates miR-24, which targets HNF4à, which is a transcription factor for miR-122 expression. Thus, upon inflammation, miR-122 can be decreased due to pSTAT3 signalling. Consequently, suppressing pSTAT3, leads to increased miR-122 expression level. We hypothesize that a small molecule that increases miR-122 activity, could be assessed as a therapeutic agent against pre-malignant and malignant liver conditions.

Conclusion: CD24, a cancer stem cell marker, is a direct target of miR-122. Thus, reducing CD24 levels with miR-122 will enable an immune attack on the hepatic cancer stem cells, and suppress cancer stem cell proliferation in the liver. We show a therapeutic benefit using anti-CD24 and pSTAT3 inhibitors.

OS-3-YI
Effect of Protease-Activated Receptor 2 inhibition by 1-Piperidinepropionic acid in lipid accumulation, inflammation and hepatocellular carcinoma development

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Background and aims: Hepatocellular carcinoma (HCC), Metabolic-Associated Steatotic Liver Disease (MASLD) and Metabolic-Associated Steatohepatitis (MASH) are major challenges in modern hepatology due to their rising incidence and the lack of specific treatments. 1-Piperidinepropionic Acid (1-PPA) is a novel inhibitor of Protease-Activated Receptor 2 (PAR2), which is involved in inflammation, lipid accumulation and tumor development. This study aims to evaluate the effect of 1-PPA on liver steatosis, inflammation and HCC development.

Method: We used a mouse model of NASH-related liver carcinogenesis to assess if 1-PPA could reduce liver steatosis, inflammation and HCC development. C57BL/6J mice transgenic overexpressing SerpinB3 (C57/TG), fed on a CDAA diet and injected with diethylnitrosamine (DEN) were divided into two groups (n=8 each) and treated with 1-PPA or placebo. HCC development was confirmed by liver histology. Microsomal triglyceride transfer protein (MTP) activity was quantified in liver tissue using a specific assay. qPCR of macrophage M2-polarization markers was also carried out. Human liver organoids were cultured with different steatogenic conditions (Oleic Acid and SB3) and treated with 1-PPA and lomitapide, an inhibitor of VLDL export. Lipid and ROS accumulation was quantified using BodiPY and MitoSOX respectively.

Results: C57/TG mice treated with 1-PPA developed a lower number of nodules (n=1.5 vs n=5, $p < 0.05$), a reduced mean tumoral mass (0.04 vs 0.1 g, $p < 0.01$) and a blunted expression of M2-polarization macrophage markers (namely ARG2, YM1, MRC1, COX2, NOX2, TNF-alpha). Serum triglycerides were significantly elevated in 1-PPA-treated mice (0.23 vs 0.16 mg/dL, $p < 0.05$), paralleling an increased MTP activity in liver tissue. Human liver organoids cultured with both Oleic Acid and SB3 showed a significant increase in lipid and ROS accumulation and treatment with 1-PPA reverted this effect which was erased by contemporary treatment with lomitapide, suggesting a role in VLDL export mediated by this molecule.

Conclusion: 1-PPA treatment reduced HCC development by reducing lipid accumulation and M2-macrophage polarization in a mouse model of NASH-induced liver carcinogenesis. 1-PPA treatment reduced lipid accumulation by stimulating VLDL formation and secretion both in a mouse model of NASH-induced liver carcinogenesis and in human liver organoids.

OS-4-YI

The role of a 13-serum-protein signature reflecting inflamed class hepatocellular carcinoma in patients receiving medical therapies

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Background and aims: The medical therapy landscape for patients with advanced hepatocellular carcinoma (HCC), is increasingly complex, with survival benefit attributed to a minority of responders, while others experience side-effects which impair quality of life. Disappointingly, immuno-oncology (IO) predictive biomarkers from other tumour types (PDL1 expression, mutational burden, microsatellite instability) are not associated with responses in HCC. Montironi et al (Gut,2023) identified a 13-serum-protein signature reflecting features of the inflamed tumour microenvironment (TME), with potential to predict differential IO treatment responses. We have explored this.

Method: 160 patients with advanced HCC receiving first line medical therapy were included, as part of the CRUK-HUNTER observational study. Best Imaging response on CT/MRI defined partial response (PR), stable disease (SD) or progressive disease (PD). 41 received Sorafenib (SD 19, PD 22), 51 nivolumab (PR 3, SD 32, PD 16) and 68 atezolizumab+bevacizumab (atezo/bev) (PR 29, SD 18, PD 21). The relative abundance of the 13-serum-proteins was measured in pre-treatment samples using a proximity extension assay (https://olink.com/). Weighted scores within each treatment group were calculated by summing each protein's z score multiplied by its corresponding response associated logistic regression coefficient.

Results: Treatment groups were similar, with few differences between responders and non-responders. Scores derived from the 13-serum-proteins were significantly associated with response in each of the 3

treatment categories. Nivolumab responders (PR/SD vs PD) showed the greatest mean score difference (1.5 vs -0.04; $p < 0.001$) and the strongest predictive performance with an area under the curve (AUC) by receiver operator characteristic (ROC) analysis, of 0.8. For sorafenib cases, the mean sore associated with SD, was 1.23 compared to -0.64 in PD ($p = 0.001$), with an AUC of 0.79. For atezo/bev cases, responders had a mean score of 1.3 vs 0.33 in non-responders ($p < 0.001$) with an AUC of 0.77. Notably, the positive and negative coefficients creating the scores within treatment categories were quite distinct (eg. VEGFR2 positive with atezo/bev response, negative for others; IL-18 negative atezo/bev, positive for others; DNER and TNFRSF21 negative for sorafenib; ADA positive for sorafenib).

Conclusion: Within each treatment category, combination scores created from the 13-protein serum proteins reflecting the inflamed class of HCC, were associated with differential responses. Individual components associated with response or progression within the categories differed. With these 13 serum proteins, there is the potential to identify the treatment type the individual is more likely to respond to. Prospective validation will be required.

OS-5-YI

A comprehensive evaluation of immune infiltration in hepatocellular carcinoma treated with liver surgery after selective internal radiation therapy

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Background and aims: Selective internal radiation therapy (SIRT) is a promising treatment for hepatocellular carcinoma (HCC) at all stages, particularly as bridging or downstaging treatment for liver resection (LR)/liver transplantation (LT), but its effect on the tumor milieu remains to be explored. This study aims to evaluate the intratumoral microenvironment and gene expression profiles in HCC patients who underwent surgery after SIRT and its impact on survival.

Method: Thirty-six HCC patients treated with SIRT followed by LR/LT at two French academic hospitals between 2014 and 2023 were included in the study. Demographic/biological/clinical data were collected. Tumor response was assessed 6-months after SIRT using mRECIST criteria. RNA sequencing was analyzed to study gene expression profiles in the intratumoral microenvironment of formalin-fixed paraffin-embedded (FFPE) surgical liver samples. Immune infiltrates were estimated using the MCP-counter method. All statistical analyses were performed using R version 4.2.0.

Results: Twenty-five patients (84% male, median age 67 years, 80% with cirrhosis) who underwent LR/LT after SIRT were included in the final analysis. Thirteen patients (52%) were BCLC-C without extrahepatic metastases, 10 (40%) BCLC-B, and 2 (8%) BCLC-A. Ten patients (40%) had a complete response (CR) 6 months post-SIRT, 12 (48%) a partial response (PR), and 3 (12%) stable disease (SD). Seventeen patients (68%) underwent LR, and 8 (32%) LT. Fourteen patients (56%) developed tumor recurrence post-surgery, and 11 (44%) died. The intratumoral microenvironment showed no significant difference in immune infiltrate between patients with and without CR. Patients with SD had significantly lower intratumoral infiltration of neutrophils (p=0.027) and myeloid dendritic cells (p=0.049) than others. In patients with CR, 28 DEGs were up-regulated and 37 down-regulated. Gene enrichment analyses revealed that patients with CR had down-expression of immune response-related signaling pathways, and non-responders showed over-expression of genes linked to a more aggressive HCC subtype. No differences in immune infiltrate were found between patients with and without recurrence. Supervised analysis identified 49 down- and 25 up-regulated genes in patients with HCC recurrence, with gene expression profiles associated with immune response. Multivariate Cox-regression analysis identified

age>60 years (p=0.039), cirrhosis (p=0.032), and the presence of satellite nodules in surgical tissue samples (p=0.012) as independent predictors of death.

Conclusion: SIRT influences the immune microenvironment in HCC, being CR associated with a down-regulated immune response. These results suggest that patients with active residual tumors post-SIRT could benefit from a combination of immunotherapy and surgery to enhance anti-tumor responses and prevent recurrence.

OS-6
Subsequent anticancer therapy analysis of the Phase 3 HIMALAYA study of tremelimumab plus durvalumab in unresectable hepatocellular carcinoma

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Background and aims: In the Phase 3 HIMALAYA study (NCT03298451), STRIDE (Single Tremelimumab Regular Interval Durvalumab) significantly improved overall survival (OS) versus sorafenib in participants (pts) with unresectable hepatocellular carcinoma (uHCC; Abou-Alfa et al. *NEJM Evid* 2022). The OS benefit with STRIDE persisted after 5 years of follow-up (OS HR 95% confidence interval [CI] vs sorafenib, 0.76 [0.65–0.89]; Rimassa et al. Presented at ESMO Congress; 13–17 September 2024; Barcelona, Spain. Oral Presentation 974MO). Assessment of subsequent anticancer therapy (SAT) use is key to understanding the long-term benefits and outcomes of STRIDE. Here, we report the assessment of SAT use at the 5-year follow-up.

Method: Pts with uHCC were randomised to STRIDE, durvalumab monotherapy or sorafenib. SAT use, time to first subsequent treatment or death (TFST) and OS in the STRIDE and sorafenib arms were assessed. Data cut-off was 1 March 2024.

Results: Median (95% CI) duration of follow-up was 62.49 (59.47–64.79) months for STRIDE and 59.86 (58.32–61.54) months for sorafenib. Of 1171 pts randomised, 168/393 (42.7%) pts in the STRIDE arm and 179/389 (46.0%) pts in the sorafenib arm received SATs, and 20/393 (5.1%) pts in the STRIDE arm and 6/389 (1.5%) pts in the sorafenib arm remained on initial study treatment. Subsequent immunotherapy use was higher in the sorafenib arm (94/389 [24.2%] pts) than the STRIDE arm (25/393 [6.4%] pts) at 60 months, while targeted therapy use was higher in the STRIDE arm (155/393 [39.4%]) than the sorafenib arm (112/389 [28.8%]); subsequent chemotherapy use was similar between the STRIDE arm (24/393 [6.1%]) and sorafenib arm (27/389 [6.9%]). Median (95% CI) TFST was longer for STRIDE versus sorafenib (8.44 [7.23–10.22] months vs 7.13 [5.98–7.95] months; HR, 0.77; 95% CI, 0.66–0.89). Median (95% CI) OS was numerically longer for STRIDE versus sorafenib in pts with SAT use (26.7 [20.7–30.2] months vs 20.9 [16.9–24.4] months; HR, 0.80; 95% CI, 0.64–1.01) and without SAT use (10.7 [8.7–14.3] months vs 8.1 [6.3–10.3] months; HR, 0.71; 95% CI, 0.58–0.88). OS benefit with STRIDE was consistent after censoring pts at initiation of any SAT (OS HR [95% CI], 0.74 [0.60–0.92]).

Conclusion: STRIDE maintained longer OS versus sorafenib, regardless of SAT use, further supporting its independent and sustained effect on pt survival. Furthermore, delayed TFST with STRIDE provides an additional measure of effectiveness for STRIDE in addition to its effect on pt survival.

OS-7
A holistic approach of systematic tumor and non-tumor biopsy during percutaneous radiofrequency ablation for hepatocellular carcinoma:diagnostic, prognostic and therapeutic impact

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Background and aims: We prospectively assess the feasibility, safety, and diagnostic/prognostic value of systematic tumor and non-tumor liver biopsies collected during radiofrequency ablation (RFA) for a first diagnosis of hepatocellular carcinoma (HCC).

Method: We included patients diagnosed with a first diagnosis of HCC between 2015 and 2021, treated with percutaneous RFA and with both tumor and non-tumor biopsies in a tertiary center. We analyzed the percentage of diagnostic tumor biopsies and correlated results of histological findings with prior diagnoses and oncological outcomes. Non-tumor liver biopsy results were compared with multidisciplinary tumor board (MTB) diagnosis of cirrhosis.

Results: 248 patients (86% male, median age 68) with 302 tumors treated by RFA were included. HCC was single in 78%, bifocal in 21%, with a median size of 24mm. 87% of the patients had cirrhosis (based on the results of the non-tumor liver biopsy done per-procedure). Bleeding occurred in 6 cases (1.9%), requiring intervention in 2, with no death. Nor low platelets-count (< 100000/mm^3) nor anticoagulation nor antiplatelet agent were associated with bleeding. Biopsies were diagnostic in 66% with positivity linked to nodule size (P<0.0001), location (P=0.04), and ultrasound visibility (P=0.004). Among the 302 biopsies, 34% were not diagnosis, 61% diagnosed HCC, 3% cholangiocarcinoma (n=3) / hepatocholangiocarcinoma (n=6), and 2% dysplastic nodules (n=7). Discrepancies between MTB and histological diagnosis occurred in 5% of cases. To note, all the cholangiocarcinoma or hepatocholangiocarcinoma diagnosed with the per-ablation biopsy were classified as LIRADS 5 by imagery reviewing. Survival was significantly shorter in patients with cholangiocarcinoma/hepatocholangiocarcinoma (P<0.001). Pathological subtypes of HCC were not otherwise specified HCC in 55%, steatohepatitic-HCC in 19%, macrotrebacular massive-HCC (MTM-HCC) in 15%, scirrhous-HCC in 6.3%, clear cell-HCC in 2.8%, and lympho-epithelioma like-HCC in 2.3%,. MTM-HCC was associated with higher rate of tumor recurrence (P=0.037). Presence of more than 30% of tumor cells at formalin-fixed paraffin-embedded samples was associated with expression of cancer-related genes at transcriptomic of the corresponding frozen samples assuring their usefulness for molecular analysis. Among non-tumor biopsies, cirrhosis was histologically confirmed in 82% of cases with a 15% discrepancy between diagnosis of cirrhosis at MTB and biopsy, 10.9% (n=27) were considered cirrhotic by MTB and identified as non-cirrhotic on the non-tumor biopsy.

Conclusion: Systematic tumor and non-tumor biopsy during RFA for a first diagnosis of HCC is feasible, safe, and has diagnostic, therapeutic, and prognostic value.

OS-8

Should liver transplantation be considered for resectable perihilar cholangiocarcinoma? Preliminary results from the TRANSPHIL french randomized controlled trial.

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Background and aims: Liver transplantation following neoadjuvant chemoradiation therapy (RT-LT) has become a standard of care for patients with unresectable perihilar cholangiocarcinoma (pCCa), offering similar, if not better, long-term outcomes than liver resection performed for resectable disease. Thus, whether RT-LT indications should extend to resectable pCCa is currently under debate.

Method: A multicenter, randomized control trial was conducted at 19 liver transplant centers in France. Patients who were aged 18-69 years with potentially resectable pCCA were randomly assigned (1:1) to either receive a conventional liver/biliary tract resection with lymph node dissection (RSX arm), or a liver transplantation preceded by chemoradiation therapy (RT-LT arm). The Mayo-Clinic selection criteria (including mass lesion <3cm, tumor not extending below the cystic duct, and no metastatic disease) were applied to all patients enrolled in the study. Endoscopic biliary drainage was performed in jaundiced patients in both arms. Based on an expected 5-year overall survival (OS) of 70% after transplantation and 30% after resection, with a power of 80% and a bilateral α-risk of 5%, 54 patients (27 in each arm) were expected to be included. We present herein the preliminary results from the intent-to-treat (ITT) population. Analyses in the per-protocol population are currently ongoing.

Results: From July 2014 to July 2019, 56 patients were screened for participation in 9 centers, of whom 40 were randomly assigned to RSX (N=20) or RT-LT arm (N=20). The study was prematurely closed because of a drop-out rate of 55% in the RT-LT arm, with only 9 patients actually receiving a liver transplant, while 19 patients (95%) from the RSX arm underwent a liver resection with curative intent. Among these 11 patients who drop-out, 10 died before the end of the study from disease progression (n=6), sepsis (n=3) and other (n=1). On an ITT basis, 5-year OS rates were 30% in both RSX and RT-LT arms. Overall, the median time from randomization to resection (N=19) or transplantation (N=9) was 36 (Q1-Q3: 20-54) and 156 days (Q1-Q3: 126 - 165), respectively. Postoperative 90-day mortality was 11% (2/19) in the RSX arm and 0% (0/9) in the RT-LT arm.

Conclusion: Despite indirect evidence from previous retrospective studies suggesting a benefit of chemoradiation therapy following by liver transplantation in patients with resectable pCCa, this first randomized control trial directly comparing this approach with conventional surgical resection calls for the utmost caution when considering liver transplantation as an option for patients with resectable disease.

OS-9-YI

Mutational analysis of bile cell-free dna in primary sclerosing cholangitis: a pilot study

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Background and aims: : Primary sclerosing cholangitis (PSC) is a chronic liver disease characterized by inflammation and fibrosis of the bile ducts conferring an increased risk of cholangiocarcinoma (CCA). However, detecting CCA early in PSC patients remains challenging due to limited sensitivity of conventional diagnostic methods, such as imaging or bile duct brush cytology during endoscopic retrograde cholangiopancreatography (ERCP). In this study, we aim to evaluate the potential of bile cell-free DNA (cfDNA) mutational analysis, termed the Bilemut assay, as a tool for CCA detection in PSC patients.

Method: A total of 63 PSC patients undergoing ERCP due to bile duct strictures were prospectively recruited. Bile samples were collected during ERCP, and cfDNA was extracted and analyzed using the Oncomine™ Pan-Cancer Cell-Free assay. A control group of 20 healthy liver donors was included for comparison. Next generation sequencing for 52 genes was performed and samples with a mutant allele frequency (MAF) ≥ 0.1% were considered Bilemut positive. Clinical data were analyzed to assess any correlation between mutational status and clinical characteristics or malignancy outcomes.

Results: cfDNA mutational analysis was successfully performed on all bile samples. Mutations were detected in 36.5% (23/63) of PSC patients, with the most frequently mutated genes being *KRAS* (65%), *GNAS* (30%), and *TP53* (17%). Comparatively, only 10% (2/20) of healthy donors showed a mutation, significantly lower than in PSC patients ($p = 0.0269$). The clinical characteristics of Bilemut-positive and Bilemut-negative patients were comparable, though there was a trend towards a lower prevalence of inflammatory bowel disease (IBD) in the Bilemut-positive group. Among PSC patients diagnosed with CCA during follow-up, 75% were Bilemut-positive, suggesting an association between mutational status and malignancy risk.

Conclusion: Mutational analysis of cfDNA obtained from bile collected from PSC patients undergoing ERCP is feasible. The routine implementation of the Bilemut assay may help identifying patients needing closer surveillance and further imaging studies.

OS-10-YI
Understanding the impact of Clinical Nurse Specialist led holistic needs assessment on patient's cancer journey and care planning for patients with Hepatocellular Carcinoma

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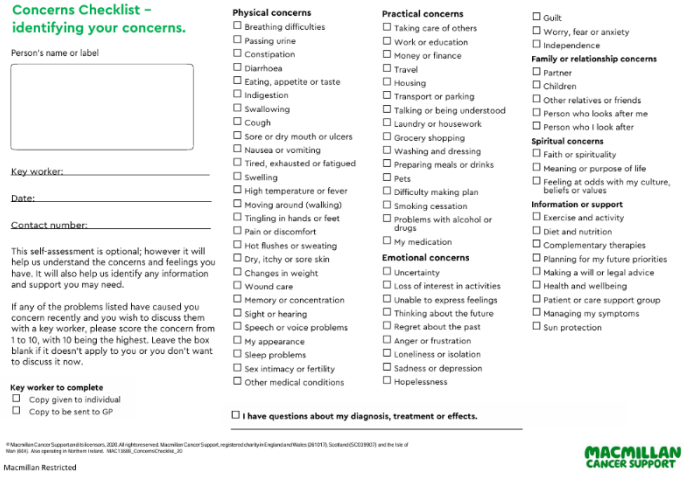
Background and aims: Within a regional Hepatocellular Carcinoma centre; the clinical nurse specialist (CNS) team incorporated a pathway to support patients and identify and address care needs using a personalised approach. Every patient at diagnosis is offered a holistic needs assessment (HNA) checklist and appointment to meet their care needs. The CNS is part of the multidisciplinary team and providing advanced practice in the management and care for patients with HCC. Our aim was to understand the unmet care needs for patients with HCC and the impact the CNS can have on this.

Method: All new patients were offered a checklist (figure 1) to complete prior to a virtual appointment to review their needs and create an individual, person centered care plan to address their needs with a CNS. These needs were analyzed over a 1 year period and collated on the my care plan platform (Macmillan). Patient feedback was gathered from 20% of patients after their care planning appointment.

Results: There are significant care needs for patients diagnosed with HCC. The 3 most common care needs identified were treatment information, transport and travel and carer needs however pain and fatigue were the highest priority of need. Fatigue score >6 (scale 1-10 with 10 being very severe) was present in >20% of diagnostic HNA. This analysis was of 146 HNA review with CNS over 1 year with 83% at diagnosis.
Further analysis at follow up HNA showed 68% reduction in practical needs alongside reduction in symptom burden score/severity. Patient feedback questionnaire after the appointment showed high satisfaction with CNS interaction and intervention.

Conclusion: The role of the clinical nurse specialist is essential to ensure unmet needs are identified and a plan to care agreed especially within HCC as there is increased symptom burden and care needs which can be improved with specialist intervention. Further analysis of needs based on treatment stage can further stratify the CNS role in the care journey.

Figure 1



Concerns Checklist – identifying your concerns.

Person's name or label

Key worker:

Date:

Contact number:

This self-assessment is optional; however it will help us understand the concerns and feelings you have. It will also help us identify any information and support you may need.

If any of the problems listed have caused you concern recently and you wish to discuss them with a key worker, please score the concern from 1 to 10, with 10 being the highest. Leave the box blank if it doesn't apply to you or you don't want to discuss it now.

Key worker to complete
- ☐ Copy given to individual
- ☐ Copy to be sent to GP

Physical concerns
- ☐ Breathing difficulties
- ☐ Passing urine
- ☐ Constipation
- ☐ Diarrhoea
- ☐ Eating, appetite or taste
- ☐ Indigestion
- ☐ Swallowing
- ☐ Cough
- ☐ Sore or dry mouth or ulcers
- ☐ Nausea or vomiting
- ☐ Tired, exhausted or fatigued
- ☐ Swelling
- ☐ High temperature or fever
- ☐ Moving around (walking)
- ☐ Tingling in hands or feet
- ☐ Pain or discomfort
- ☐ Hot flushes or sweating
- ☐ Dry, itchy or sore skin
- ☐ Changes in weight
- ☐ Wound care
- ☐ Memory or concentration
- ☐ Sight or hearing
- ☐ Speech or voice problems
- ☐ My appearance
- ☐ Sleep problems
- ☐ Sex intimacy or fertility
- ☐ Other medical conditions

Practical concerns
- ☐ Taking care of others
- ☐ Work or education
- ☐ Money or finance
- ☐ Travel
- ☐ Housing
- ☐ Transport or parking
- ☐ Talking or being understood
- ☐ Laundry or housework
- ☐ Grocery shopping
- ☐ Washing and dressing
- ☐ Preparing meals or drinks
- ☐ Pets
- ☐ Difficulty making plan
- ☐ Smoking cessation
- ☐ Problems with alcohol or drugs
- ☐ My medication

Emotional concerns
- ☐ Uncertainty
- ☐ Loss of interest in activities
- ☐ Unable to express feelings
- ☐ Thinking about the future
- ☐ Regret about the past
- ☐ Anger or frustration
- ☐ Loneliness or isolation
- ☐ Sadness or depression
- ☐ Hopelessness
- ☐ Guilt
- ☐ Worry, fear or anxiety
- ☐ Independence

Family or relationship concerns
- ☐ Partner
- ☐ Children
- ☐ Other relatives or friends
- ☐ Person who looks after me
- ☐ Person who I look after

Spiritual concerns
- ☐ Faith or spirituality
- ☐ Meaning or purpose of life
- ☐ Feeling at odds with my culture, beliefs or values

Information or support
- ☐ Exercise and activity
- ☐ Diet and nutrition
- ☐ Complementary therapies
- ☐ Planning for my future priorities
- ☐ Making a will or legal advice
- ☐ Health and wellbeing
- ☐ Patient or care support group
- ☐ Managing my symptoms
- ☐ Sun protection

☐ **I have questions about my diagnosis, treatment or effects.**

OS-11-YI

Modulating Wnt/"beta"-catenin pathway activity as a potential chemosensitive strategy in cholangiocarcinoma

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Background and aims: A critical limitation in the treatment of cholangiocarcinoma (CCA) patients is their poor response to chemotherapy due to the existence in tumor cells of different mechanisms of chemoresistance (MOC), leading to a marked multidrug resistance (MDR) phenotype. This has been associated in some cancers with impaired signaling pathways, such as that involving Wnt/”beta”-catenin, which is hyperactivated in CCA and plays an essential role in cell proliferation, migration and angiogenesis.

The aims are to elucidate the role of the Wnt/”beta”-catenin pathway in the MDR phenotype of CCA and to evaluate the usefulness of novel therapeutic strategies based on combining Wnt/”beta”-catenin pathway inhibition with antitumor drugs.

Method: *In vitro* assays were performed using intrahepatic (CC-LP1, CC-SWI and HuCCT1) and extrahepatic CCA cell lines (EGI-1 and TFK-1) treated with Wnt/”beta”-catenin pathway inhibitors (C59, XAV939 or PRI724) to evaluate, by RT-qPCR and WB, expression of Wnt target genes and genes involved in MDR. Taqman Low-Density Arrays (TLDAs) were used to measure mRNA expression of ~100 genes involved in several MOC. Following 72h of treatment with Wnt/”beta”-catenin inhibitors, both alone or in combination with antitumor drugs (cisplatin, gemcitabine, and 5-FU), cell viability was determined by MTT test. SynergyFinder was used to evaluate the synergistic potential of each tested drug combination. CCA was developed in rats by administration of thioacetamide (TAA) for 26 weeks in drinking water; these animals were treated with PRI724 alone or with 5-FU to evaluate the antitumor effect.

Results: Treatment with C59, XAV939 or PRI724 in CCA cell lines inhibited the activity of the Wnt/”beta”-catenin pathway, as demonstrated by downregulation of target genes *BIRC5*, *AXIN2,* and *MYC* (RT-qPCR and WB). Inhibition of the Wnt/”beta”-catenin pathway resulted in expression changes affecting several genes included in the resistome, such as genes encoding drug uptake transporters (*SLC*), and genes involved in the apoptosis/survival balance. *In vitro* assays showed that combined treatment using the Wnt/”beta”-catenin inhibitor PRI724 with antitumor drugs (cisplatin or 5-FU) induced an additive effect by reducing cell proliferation. Similarly, *in vivo* assays in rats with CCA revealed that the treatment with the combination of PRI724 and 5-FU showed a significant reduction in tumor size compared to animals receiving the vehicle.

Conclusion: Inhibition of the Wnt/”beta”-catenin signaling pathway induces chemosensitization of CCA cells by changing the expression of several MOC genes. The use of therapeutic strategies combining Wnt/”beta”-catenin pathway inhibition with chemotherapy may provide a pharmacological advantage in treating CCA patients.

OS-12-YI

Sushi domain containing 2 upregulation in fibrotic Liver Disease: Potential role in hepatocellular carcinoma progression through Interaction with galectin-1

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Background and aims: Hepatocellular carcinoma (HCC) is a major global health concern and ranks as the third leading cause of cancer-related deaths. Chronic liver damage induces an inflammatory state that accelerates fibrosis, potentially progressing to cirrhosis and increasing the risk of HCC development in affected individuals. Studies have shown that Galectin-1 (Gal-1) is overexpressed in HCC and plays a role in tumour invasion, with a possible association with Sushi Domain Containing 2 (SUSD2). SUSD2 is a transmembrane protein necessary for transporting Gal-1 to the cell membrane, where it triggers T-cell apoptosis. However, the source and role of SUSD2 in HCC remain unclear. We hypothesised that SUSD2 is upregulated in chronic liver disease (CLD), potentially through its interaction with Gal-1, creating a pro-tumorigenic microenvironment that further drives the development of HCC.

Method: To investigate the expression and localization of SUSD2 and Gal-1 in CLD and HCC, immunohistochemistry and immunofluorescence were performed on paraffin-embedded liver tissue samples. Wide-field and confocal microscopy images were processed and analysed using ImageJ and Zeiss software. Immunocytochemistry for SUSD2 was carried out on isolated primary human fibroblasts, and quantitative PCR (qPCR) was used to explore the regulation of SUSD2 and Gal-1 gene expression. To examine the influence on mesenchymal markers and Gal-1, SUSD2 expression was reduced through siRNA-mediated knockdown.

Results: Analysis of single-nucleus RNA sequencing data from CLD samples identified SUSD2 expression associated with mesenchymal cell populations, particularly fibroblasts. Immunohistochemical staining further validated this finding, showing that while SUSD2 protein was predominantly localized to vascular structures in donor liver, it was notably upregulated and displayed highly specific staining within fibrotic scars in CLD. Furthermore, co-localization of SUSD2 and Gal-1 was observed in these areas, suggesting a potential interaction within the fibrotic microenvironment. SiRNA-mediated knockdown of SUSD2 resulted in the downregulation of Gal-1 expression.

Conclusion: Fibroblast-associated SUSD2 contributes to the dense stromal network in HCC and may interact with Gal-1 to support a pro-tumorigenic microenvironment that promotes HCC progression. Targeting SUSD2 and its potential interactions could represent a novel therapeutic strategy to disrupt fibrosis and inhibit tumour development.

OS-13

Preliminary results on the utility of the GAAD algorithm for hepatocellular carcinoma screening in a prospective cohort of at-risk patients

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Background and aims: Current EASL Guideline recommends ultrasound (US) screening for hepatocellular carcinoma (HCC) in at-risk patients and considers addition of alpha-fetoprotein (AFP) to be suboptimal. The GAAD/GA[L]AD algorithms (Gender, Age, AFP, [AFP-L3], and Des-gamma-carboxyprothrombin [DCP or PIVKA-II]) show promising results in diagnosing HCC, (AUROC > 90%). There are no studies in screening.

Method: Prospective longitudinal study initiated in February/2023; recruitment now closed. Patients enrolled according to 2018 HCC EASL Guideline recommendations. Semi-annual US (Canon Aplio i800) was performed with concurrent measurement of AFP, AFP-L3, and PIVKA (Cobas E-601), and subsequent calculation of Elecsys GAAD and GALAD (Roche Dialog; normal values < 2.57 and < 2.47, respectively). US categorised according to US LI-RADS 2018. Patient follow-up: semi-annually if US1 (no suspicious lesions) and GAAD < 2.57; quarterly if US2 (suspicious nodule < 1 cm) or GAAD ≥ 2.57; and further evaluated with CT/MRI/cytology if US3 (suspicious nodule > 1 cm or portal vein thrombosis). HCC diagnosis according to EASL 2018. Patients on Warfarin, other causes of vitamin K deficiency, or Child-Pugh C stage were excluded.

Results: We included 422 patients: 63 years, 68 % male, 81.5 % cirrhosis, 19.5 % non-cirrhotic hepatitis B. Aetiologies: Hepatitis C 36 %, alcohol 29 %, metabolic 25 %. After nearly two years of follow-up, 18.5 % US categorised as US2/US3; 18.5 % and 19.7 % had altered GAAD and GALAD, respectively. To date, 12 HCC tumours diagnosed: 75 % BCLC-0/A, 25 % BCLC-B/C. In the total HCC, GAAD performance was: sensitivity (Se) 91.7 %, specificity (Sp) 83.7 %, positive predictive value (PPV) 14.3 %, negative predictive value (NPV) 99.7 %, AUROC 0.96 ($p < 0.001$); for GALAD: Se 91.7 %, Sp 82.4 %, PPV 13.4 %, NPV 99.7 %, AUROC 0.96 ($p < 0.001$). Performances of AFP, AFP-L3, and PIVKA alone were inferior, with AUROC 0.78, 0.76, and 0.89, respectively. In the subset of patients with HCC BCLC-0/A stage, GAAD performance was: Se 88.9 %, Sp 83.7 %, PPV 10.7 %, NPV 99.7 %, AUROC 0.95 ($p < 0.001$); for GALAD: Se 88.9 %, Sp 82.4 %, PPV 10.1 %, NPV 99.7 %, AUROC 0.94 ($p < 0.001$). Both a GAAD value < 2.57 combined with US1/US2 categories or an isolated GAAD < 2.12 achieved 100 % NPV.

Conclusion: Our preliminary results in a prospective HCC screening cohort suggest high performance for GAAD and GALAD algorithms. The simpler GAAD demonstrates similar efficacy to GALAD.

OS-14-YI

Novel “3-in-1” blood metabolomic test for the early diagnosis and risk stratification of primary sclerosing cholangitis and cholangiocarcinoma

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Background and aims: Primary sclerosing cholangitis (PSC) is a chronic cholestatic disease that confers high risk of developing cholangiocarcinoma (CCA). Up to 20% of patients with PSC develop CCA during their lifetime, which constitutes the primary cause of premature death in this population. Current diagnostic modalities for the early detection of PSC and CCA are suboptimal and new predictive and diagnostic non-invasive approaches are urgently needed. Here, we aimed to investigate serum metabolite biomarkers for PSC and PSC-CCA.

Method: This multicentre international study included 434 serum samples from 13 centres in eight countries worldwide. The cohort comprised patients with PSC (n=216); PSC without clinical evidences of malignancy at sampling who developed CCA during follow-up (between 2 months and 8 years before diagnosis; PSC-to-CCA; n=28); PSC with concomitant CCA (PSC-CCA;n=97); ulcerative colitis

(UC;n=14); and healthy individuals (n=79), divided in discovery (60%) and validation cohorts (40%). Serum metabolomics was evaluated by ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS) and the accuracy of single candidate metabolite biomarkers was further assessed. Machine learning was used to generate the best diagnostic and predictive algorithms.

Results: Fifty metabolites associated with PSC diagnosis independent of age, biological sex and the presence of liver cirrhosis or UC. A model combining 13 metabolites differentiated patients with PSC from healthy controls with 98% accuracy both in discovery and validation cohorts. Furthermore, 57 metabolites were altered in patients with PSC-CCA compared with PSC, independent of age, biological sex, cirrhosis and anatomical CCA subtype. A model combining 13 of these metabolites accurately confirmed the presence of CCA in patients with PSC in the discovery and validation cohorts, with area under the curve (AUC) values of 0.91 and 0.90, respectively, particularly identifying patients with early tumor stages (0-II; AUC=0.930), being superior to CA19.9 (AUC=0.646). Noteworthy, the diagnostic capacity of this model was retained (AUC=0.92) when considering only patients with low serum CA19-9 levels (i.e. false-negative test result). Finally, a model including seven metabolites allowed the prediction of CCA development in patients with PSC before any clinical evidence of cancer, with a positive predictive value (PPV) of 83% and 73% in the discovery and validation cohorts, respectively.

Conclusion: The “3-in-1” metabolomic test is a useful non-invasive tool that allow the diagnosis of PSC and early PSC-CCA, as well as to predict CCA development. Implementation in clinical practice may improve risk stratification and follow-up in patients with PSC, facilitating personalized surveillance, early diagnosis and prioritize therapeutic decisions.

OS-15-YI

Exploring the tumor immune microenvironment in cholangiocarcinoma: a spatial profiling using multiplex immunohistochemistry

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Background and aims: In cholangiocarcinoma (CCA), immune cells and immune checkpoints (ICs) play a crucial role in the tumor immune microenvironment (TIME), potentially influencing responses to anti-cancer treatments. Spatial phenotyping of TIME has become a powerful tool for assessing how immune cells are distributed and interact within solid tumors. These analyses provide insights into the heterogeneity of CCA and can reveal specific immune landscapes associated with different patient outcomes. In our study, we aim to identify potential biomarkers and develop a more stratified strategy for applying immunomodulatory therapies in patients with extrahepatic cholangiocarcinoma (eCCA).

Method: A total of 100 resected and clinically annotated eCCAs were included. Multiplex immunohistochemistry (mIHC) and multispectral imaging techniques (Akoya Biosciences) were used for detailed mapping of immune cells, including T cells, B cells and macrophages, as well as the expression patterns of immune checkpoints such as PD-1, PD-L1, CTLA-4, T cell immunoreceptor with Ig and ITIM domains (TIGIT) and lymphocyte-activation gene 3 (LAG-3). High-resolution images obtained from regions of interest (ROIs) were analyzed using machine learning processes for quantitative results and spatial profiling.

Results: Intra- and intertumoral heterogeneity were evaluated by dividing tissue sections into distinct regions. Our analysis revealed that tertiary lymphoid organs (TLOs) were detected in 76% of patients, with relatively high T cell infiltration (26%), while the tumor core showed a low presence of immune cells (< 3%), suggesting an immunosuppressive microenvironment. Among the total T cell population, approximately 10% expressed immune checkpoints, with over 90% of all IC+ T cells exhibiting single positivity. PD-L1 was the most frequent IC (38%) in the tumor core, while PD-1 (28%) and TIGIT (26%) were the most abundant in the TLOs. In addition, neighborhood analysis, such as distances between various cell types, was also performed. Ongoing investigations aim to establish spatial and clinical correlations between immune cell infiltration patterns and immune checkpoint expression levels.

Conclusion: Our study of the TIME in primary extrahepatic cholangiocarcinoma described the spatial architecture and emphasized the critical role of intertumoral heterogeneity in shaping the immune landscape. We observed distinct patterns of immune cell infiltration, indicating complex dynamics within the TIME. The presence of immune checkpoints on T cells suggested potential mechanisms of immune evasion that may impact treatment outcomes. By integrating quantitative analysis with advanced spatial profiling, our findings provide valuable insights that could improve prognostic accuracy and enhance predictions of treatment response to immunomodulatory therapies in eCCA patients.

POSTER ABSTRACT PRESENTATIONS

Basic Science

PO1-01
Selective targeting of class I histone deacetylases with new derivatives of ursodeoxycholic acid as a novel treatment strategy for bile duct cancer

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Background and aims: Cholangiocarcinoma (CCA) includes a heterogeneous group of biliary tumors characterized by poor prognosis. Available systemic therapies for patients with advanced CCA are merely palliative, attesting the need to find novel effective approaches. Altered expression and/or function of certain epigenetic regulators such as histone deacetylases (HDACs) has been closely related to the development and progression of different types of cancer, including CCA. We have previously developed a new family of HDAC6 selective inhibitors derived from ursodeoxycholic acid (UDCA) that showed preferential enterohepatic biodistribution after oral administration (Caballero-Camino, FJ, *et al*. *Hepatology* 2021). Here, we hypothesize that selectively targeting the deregulated HDAC isoforms in CCA with novel UDCA-derived HDAC inhibitors could be a valuable epigenetic-based therapeutic strategy for CCA.

Method: The expression levels of the different HDACs were analyzed in tumor tissue samples from 4 international cohorts of patients with CCA. Advanced computational methods were applied to design and synthesize UDCA-derived drug candidates. Therapeutic potential of the lead compound was further characterized in experimental models of naïve and chemoresistant CCA, including human tumor spheroids and murine CCA organoids.

Results: Class I HDACs –1, 2 and 8– were found overexpressed in human CCA tumors from 4 cohorts of patients with CCA compared to control tissues. Computationally-aided rational design yielded 9 UDCA-derived drug candidates for selective targeting class I HDACs. Among them, the lead compound (Epi-UDCA) was selected based on class I HDAC inhibitory potency and selectivity on enzymatic assays. In CCA cell lines, Epi-UDCA displayed marked antiproliferative capacity at low doses (2 μM) and strong apoptotic induction at higher doses (10 μM). Epi-UDCA halted the growth of 3D human CCA spheroids and murine tumor organoids, promoting cancer cell death. Importantly, low doses of Epi-UDCA (2 μM) halted the invasion capacity of human CCA cells in extracellular matrix (ECM)-coated transwell migration assays. Proteomic analysis of CCA cells exposed to Epi-UDCA (2 μM) revealed significant changes in the expression of 396 proteins directly involved in important cancer-related processes such as cell cycle progression, angiogenesis, ferroptosis, and transcriptional regulation by tumor suppressor protein p53, among others. Finally, Epi-UDCA displayed strong antitumoral capacity against cisplatin-resistant CCA cells and spheroids, while combined administration of Epi-UDCA with cisplatin re-sensitized these cells to chemotherapy.

Conclusion: The unique properties of Epi-UDCA, a novel UDCA-derived class I selective HDAC inhibitor, provide this drug with promising therapeutic potential for the treatment of naïve and cisplatin-resistant cancers, including CCA.

PO1-02
Spatial transcriptomic profiling reveals distinct stromal and clonal dynamics in liver cancer

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Spatial transcriptomic profiling reveals distinct stromal and clonal dynamics in liver cancer

Background and aims: Tumor heterogeneity poses a significant challenge for cancer prevention and therapy, particularly in liver cancer (LC), which ranks as the second leading cause of cancer mortality worldwide. The two major histologic subtypes, hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA), exhibit considerable inter- and intra-tumor diversity, influenced by genetic, biological, and radiopathologic variations. Recent single-cell omics studies have advanced our understanding of these complexities, particularly in relation to stromal and immune cell infiltration within tumors. However, the loss of spatial and morphological data in these approaches limits their utility in fully exploring the tumor microenvironment. To address this, we conducted spatial transcriptomics (ST) analysis on samples from 48 LC patients, encompassing histologic features such as nodule-in-nodule HCC (NIN-HCC), small duct iCCA (SD-iCCA), and large duct iCCA (LD-iCCA).

Method: Formalin-fixed paraffin-embedded (FFPE) tissue specimens from 48 liver cancer patients (26 HCC and 22 iCCA cases) were subjected to ST sequencing using the 10X Genomics Visium platform.

Results: We identified a unique stromal cell population characterized by COL1A1 expression, which was more prominent in LD-iCCA than in other LC types, indicating that their abundance in tumor tissue is associated with unfavorable prognostic outcomes. Additionally, we identified spatial neighborhoods between COL1A1-expressing stromal cells and malignant cells. In NIN-HCC cases, we observed two nodules formed over time within the same tissue, with the later-formed inner nodule exhibiting poorer differentiation than the outer nodule. This enabled us to track the progression and differentiation process of HCC. Our spatial approach allowed us to identify key molecular processes associated with HCC progression, such as epithelial-to-mesenchymal transition and cell cycle regulation.

Conclusion: Our spatial transcriptomic analysis reveals distinct stromal and clonal dynamics in LC, uncovering prognostic biomarkers and molecular processes that may inform targeted therapeutic strategies.

PO1-03
Activated Gold-Nanoparticles enhance PD-1 blockade and boost cytotoxic T cell anti-tumor responses in cholangiocarcinoma

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Background and aims: During the last decade, immunotherapy has revolutionized cancer treatment. However, its efficacy in solid tumors, such as cholangiocarcinoma (CCA), is often limited by the immune-resistant tumor microenvironment (TME). In CCA, the dense extracellular matrix (ECM) and cancer-associated fibroblasts (CAFs) form a fibrotic stroma that acts as both a physical and biological barrier to immune cell infiltration, thereby promoting immunosuppression. Overcoming these barriers is crucial to fully unleash the therapeutic potential of immunotherapy in fibrotic tumors like CCA.

Method: We explored photothermal therapy (PTT) in combination with gold-iron oxide nanoparticles (GIONFs) and PD-1 checkpoint inhibition in preclinical CCA models.
Localized laser irradiation, as PTT, was used to activate the nanoheaters (GIONFs), creating a targeted thermal effect, remodeling the ECM and reducing tumor stiffness, boosting immune cell infiltration.
This mechanical alteration of the TME was paired with PD-1 blockade to enhance T-cell activation and reduce the immunosuppressive effects of CAFs. Alterations in immune activity, ECM stiffness, CAF modulation, and tumor progression were analyzed.

Results: Our results demonstrated that the PTT combination and GIONFs effectively remodeled the ECM, reducing stiffness and breaking down barriers to immune cell infiltration. This remodeling significantly increased cytotoxic T-cell (CD8a+) infiltration and activation. The combination with PD-1 blockade further enhanced immune responses by boosting T-cell activity and reducing CAF-mediated immunosuppression. Preclinical models showed marked reductions in tumor burden and improved tumor control, surpassing the effects of individual therapies. Additionally, molecular analyses revealed upregulation of antigen presentation pathways, amplifying immune-mediated tumor clearance.

Conclusion: These findings suggest that targeting the ECM to reduce physical barriers and combining it with immune checkpoint inhibition, offers a promising therapeutic strategy to overcome immune resistance in fibrotic tumors like CCA. This approach reshapes the TME, enhances immune cell infiltration, and improves immunotherapy efficacy. By addressing both the mechanical and immune barriers, this combination therapy may provide a new avenue for treating fibrotic solid tumors.

PO1-04-YI
An immature neutrophil signature associated with response to transarterial chemoembolization

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Background and aims: While transarterial chemoembolisation (TACE) is commonly administered to patients with intermediate stage HCC, its effectiveness is variable, with non-responders often unsuitable for subsequent medical therapy, owing to deterioration in liver function or performance. Patient stratification with predictive biomarkers, avoiding harm, would be valuable. We aim to develop a liquid biopsy tool as cheap, quick, and non-invasive predictive biomarker guiding selection for TACE.

Method: Patients treated with 1st line TACE were recruited to the Hepatocellular Carcinoma Expediter Network (HUNTER). The stage matched (single tumour < 5cm or 3 less than 3cm, Childs Pugh A liver function) cohort of 55 patients included 24 with complete response at 1 year and 31 with progression. PAXgene peripheral blood mononuclear cells (PBMCs) obtained pre-treatment were analysed by bulk RNA-sequencing, with candidate biomarkers validated at the protein level by standard flow cytometry and ImagestreamX.

Results: Differential expression analysis revealed a highly significant ($p < 0.001$) upregulation of 7 transcripts in TACE responders associated with immature neutrophils, including *LTF, CD177,* and *MMP8*. Pathway analysis highlighted neutrophil degranulation, as well as fibrosis-related pathways, platelet activation and extracellular matrix organization as upregulated in TACE responders. CibersortX deconvolution confirmed a significant upregulation of neutrophil-associated transcripts in the responder group ($p < 1.0 \times 10^{-15}$). *C4BPA,* an inactivator of the inflammatory classical complement pathway and putative suppressor of neutrophil activation, was found highly upregulated in neutrophils non-responders.

Conclusion: A transcriptionally active immature neutrophil phenotype is associated with response to TACE. This phenotype can be detected by neutrophil flow cytometry. Prospective validation of this candidate predictive tool is required and is ongoing. C4BPA has potential as a negative predictive biomarker and potential therapeutic target.

PO1-05-YI

BAP1 mutations reveal a subgroup of BTC with distinct prognosis and benefit from adjuvant therapy: a retrospective biomarker analysis externally validated in a reconstructed global cohort

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Background and aims: Biliary tract cancer (BTC) is an aggressive malignancy with poor prognosis. For patients with operable BTC, there is a lack of biomarkers predicting relapse risk and benefit from adjuvant therapy. We aimed to evaluate molecular phenotypes associated with early-stage BTC and their utility for patient stratification.

Methods: We retrospectively collected data from BTC patients who underwent tumor profiling at Humanitas Research Hospital (HU cohort). Associations between gene alterations and BTC stage (AJCC/TNM) were assessed using Fisher's exact test/Chi-square test and univariate logistic regression, with significance set at $p < 0.05$ (two-sided). Gene alterations associated with stage I BTC were further evaluated for their impact on overall survival (OS) in univariable and multivariable models. Findings from the HU cohort were validated in an external BTC dataset (global BTC cohort) with clinico-genomic annotations (from cBioPortal and Dong L, Cancer Cell, 2022).

Results: The HU cohort included 291 BTC patients (85% intrahepatic cholangiocarcinoma; 11% extrahepatic cholangiocarcinoma; 4% gallbladder cancer) profiled between 2014-2024. Median age was 65 years (21–86), with 51% male. Among patients with operable BTC (51%; 26% stage I, 36% stage II, 30% stage III), 32% received adjuvant therapy. Only BAP1 mutations were significantly associated with stage I vs. II-IV (34% vs. 16%; OR 2.81, 95% CI 1.15-6.68, $p = 0.023$). BAP1 mutations enrichment in stage I was confirmed in univariate logistic regression in both the HU (OR 2.82, 95% CI 1.16-6.65, $p = 0.019$) and global BTC cohorts (16% vs. 9%; OR 2.14, 95% CI 1.21-3.68, $p = 0.007$). BAP1 mutations were also linked to prolonged OS in both HU (HR 0.27, 95% CI 0.11-0.68, $p = 0.005$) and global BTC (HR 0.68, 95% CI 0.50-0.92, $p = 0.011$) cohorts. Pooled HU and global BTC cohorts ($n = 1013$) confirmed significantly improved OS in BAP1-mutant BTC in both univariable (HR 0.57, 95% CI 0.43-0.76, $p < 0.001$) and multivariable models (HR 0.46, 95% CI 0.23-0.90, $p = 0.024$), adjusted for stage (I vs. II-IV) and adjuvant therapy. Notably, BAP1 mutations correlated with longer OS only in patients not receiving adjuvant therapy (HR 0.45, 95% CI 0.22-0.92, $p = 0.026$), particularly in earlier stages (untreated stage I-II vs. III-IV; HR 0.10, 95% CI 0.01-0.74, $p = 0.006$).

Conclusion: BAP1 mutations are enriched in early-stage BTC and identify a subgroup with better outcomes and possibly reduced benefit from adjuvant therapy. Prospective validation is warranted for tailored adjuvant therapy decisions.

PO1-06-YI
Targeting UBE2I-mediated protein hyper-SUMOylation halts cholangiocarcinoma progression and rewires the tumor-stroma crosstalk

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Background and aims: Cholangiocarcinoma (CCA) includes a diverse range of biliary malignant tumors characterized by dismal prognosis. Alterations in post-translational modifications contribute to abnormal protein dynamics, cellular disturbances, and disease. In this study, we investigated the role of SUMOylation in CCA progression and its potential as a therapeutic target.

Method: Using a combination of human CCA cells and primary tumours, we analysed the levels and function of protein SUMOylation, identifying deregulated SUMOylated proteins by immunoprecipitation and mass spectrometry analysis. The response to genetic (*UBE2I*-knockdown) or pharmacological (ML792 and SAMe) inhibition of SUMOylation was assessed *in vitro* and in two *in vivo* models of CCA. Co-culture experiments between wild-type or UBE2I-knockdown cancer cells and CCA-derived cancer-associated fibroblasts (CAFs) and human endothelial cells were carried out to unveil the impact of SUMOylation in the crosstalk between CCA cells and the TME.

Results: Analysis of human CCA cells and primary tumors revealed increased expression and activity of the SUMOylation machinery, correlating with unfavorable clinical outcomes. Critically, we found that the majority of SUMOylated proteins deregulated in CCA, identified after immunoprecipitation and mass spectrometry analysis, are implicated in cell proliferation, survival, or cellular homeostasis. The response to genetic (UBE2I-knockdown) or pharmacological (ML792 and SAMe) inhibition of SUMOylation was assessed in vitro and in two in vivo models of CCA. Depleting SUMOylation effectively halted tumorigenesis in subcutaneous and oncogene-driven models of CCA in vivo. Furthermore, the genetic and pharmacological inhibition of SUMOylation reduced CCA cell proliferation and hindered colony formation and spheroid growth in vitro. Co-culture experiments between wild-type or UBE2I-knockdown cancer cells and CCA-derived cancer-associated fibroblasts (CAFs) and human endothelial cells were then carried out to unveil the relevance of SUMOylation in the crosstalk between CCA cells and the tumor microenvironment (TME). Importantly, experimental depletion of SUMOylation encompasses a profound impact on the establishment of the TME as it arrests the growth of CAFs and vascular cells.

Conclusion: Aberrant protein SUMOylation contributes to CCA progression by promoting cell survival and proliferation, and contributes to the conformation of the TME. Impairing SUMOylation halts CCA

growth and, thus, may represent a potential novel therapeutic strategy for patients with CCA for whom current targeted therapies are limited.

PO1-07-YI
In-situ vaccination with Flt3L elicits immune responses against hepatocellular carcinoma and synergizes with checkpoint blockade therapy

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Background and aims: Systemic therapies, including checkpoint blockade, have limited efficacy in hepatocellular carcinoma (HCC). In orthotopic mouse models of fibrosis-induced HCC and steatosis-induced HCC, we developed an *in-situ* vaccine (ISV) to recruit and activate classical dendritic cells type 1 (cDC1) and enhance their cross-presentation of tumor-associated antigens to cytotoxic T lymphocytes (CTL).

Method: Male C57BL/6J mice received a single injection of N-nitrosodiethylamine (DEN), followed by either repeated intraperitoneal carbon tetrachloride (CCl_4) injections or Western Diet feeding to induce orthotopic HCC with underlying chronic liver disease. The *ISV* combined systemic injections of the DC growth factor Fms-like tyrosine kinase 3 ligand (Flt3L), a Fas agonist, and two adjuvants (agonistic anti-CD40 or polyIC). Combination with checkpoint blockade was investigated. Immune responses were evaluated using spectral flow cytometry, multiplex immunofluorescence and bulk RNA sequencing of sorted intratumoral leucocytes. Tumor growth was analyzed with longitudinal magnetic resonance imaging. Signatures of cDC1 were explored in the TCGA-HCC dataset and the immune microenvironment of resected human HCC was investigated with multiplex immunofluorescence.

Results: Injections of Flt3L significantly expanded intratumoral and systemic cDC1 numbers. Injection of both adjuvants induced upregulation of markers of antigen cross-presentation and co-stimulation on Flt3L-recruited cDC1s, indicating maturation. Intratumoral CTL acquired an antigen-experienced, activated, effector memory phenotype after vaccination. Mice treated with ISV displayed significantly delayed tumor growth and prolonged survival compared to non-vaccinated animals. While the systemic depletion of CD4+ T cells and NK cells did not impair vaccine efficacy, CD8+ T cell depletion or absence of cDC1 cells abrogated the antitumor efficacy of the ISV. Due to high checkpoint expression in the immune microenvironment (mainly PD-1 and PD-L1), ISV was combined with anti-PD1 and anti-PD-L1 treatment. While tumors did not respond to checkpoint monotherapy, the combination of ISV with anti-PD1 showed synergistic effects and significantly prolonged median survival. In the TCGA-HCC dataset, a higher expression of cDC1 signatures was associated with the expression of adaptive immune response genes and with higher progression free 24-month survival, independent of stage, grading, and resection status.

Conclusion: Flt3L-based in situ vaccination elicits anti-tumor immunity and induces response to checkpoint blockade in previously unresponsive HCC tumors.

PO1-08-YI
Therapeutic potential of targeting Processing-bodies in Metabolic Dysfunction-Associated Steatohepatitis and hepatocellular carcinoma

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Background and aims: Processing-Bodies (P-bodies) are small cytoplasmic compartments composed of untranslated mRNAs and proteins involved RNA decay (i.e., decapping enzymes, deadenylases, exonucleases). P-bodies control the expression of a wide range of genes associated with inflammatory, metabolic, and cancer-related processes. Accordingly, alterations of P-bodies assembly or P-bodies components contribute to the development of a wide range of diseases, including cancers. Although previous works have reported that some P-bodies-related proteins (e.g., TTP, CNOT6L) are involved in metabolic dysfunction-associated steatotic liver disease (MASLD) and hepatocellular carcinoma (HCC), the role of P-bodies remains largely unexplored. Herein, we aim at characterizing the role of P-bodies in MASLD and HCC.

Method: The expression of specific P-bodies markers (EDC4, DCP1A, DDX6, TTP) was investigated in liver tissues from patients or mouse models of MASLD, HCC. The impact of different stress (oxidative stress, lipotoxicity) and anti-cancerous drugs (sorafenib) on P-bodies assembly was investigated by immunofluorescence staining of P-bodies proteins (EDC4) in hepatic cancer cells and primary hepatocytes. Loss of function analyses (siRNA) were performed in a panel of hepatic cancer cell lines to assess the impact of P-bodies proteins deficiency (EDC4) on cancer cells proliferation, survival and sensitivity to stress. In vivo experiments were carried out to decipher the impact of TTP or EDC4 silencing (i.e., AAV8 encoding shRNAs) in MASLD development, in mice fed a CSAA (choline supplemented L amino acid defined) or a CDAHFD (choline deficient L amino acid defined, high-fat diet).

Results: The expression of TTP is downregulated in HCC as compared to adjacent hepatic tissues. Although EDC4 is heterogeneously expressed in HCC, the high expression of EDC4 correlates with a poor clinical outcome. Interestingly, an increase of P-bodies number was observed in sorafenib/hydrogen peroxide-treated cells, thus suggesting that P-bodies assembly is part of a stress response. Moreover, EDC4 is upregulated in sorafenib-resistant HCC patients as compared to responders. EDC4 silencing reduces P-bodies number and hinders hepatic cancer cells proliferation and viability, thus suggesting a tumor promoting function. Preliminary findings suggest that EDC4 silencing in vivo markedly reduces hepatic steatosis in mice fed a CSAA or a CDAHFD.

Conclusion: Our study suggest that P-bodies assembly contribute to the development of MASLD and HCC and may represent a resistance mechanism against harmful conditions and sorafenib. Targeting P-bodies may therefore represent an efficient therapeutic approach for the management of MASLD and HCC.

PO1-09-YI
Role of the splicing factor poly(rC)-binding protein 2 in cholangiocarcinoma malignancy and chemoresistance

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Background and aims: Poly(rC)-binding protein 2 (PCBP2), a splicing factor member of the hnRNP family of regulatory proteins, was reported to be altered in different types of cancer, affecting tumorigenesis and chemoresistance. However, its relevance in cholangiocarcinoma (CCA) is yet unknown. Thus, this study aimed to elucidate the role of PCBP2 in developing CCA malignant characteristics and drug resistance.

Method: PCBP2 expression was investigated in silico in CCA (n = 36) and adjacent non-tumor tissues (n = 9) using data from TCGA and determined by TLDA and immunohistochemistry in samples from intrahepatic CCA (iCCA; n = 13), extrahepatic CCA (eCCA; n = 10) and adjacent non-tumor tissue (n = 9), obtained at the Salamanca University Hospital. Human healthy cholangiocytes (MMNK-1) were also included as a control. Besides CCA cells (EGI-1) and hepatocellular carcinoma derived cells (Huh7) were used as in vitro models. Lentiviral vectors carrying shRNA against PCBP2, and empty vectors (Mock) were generated to transduce liver cancer cells. PCBP2 silencing was confirmed by RT-qPCR and WB. Transcriptomic analysis of these cells was carried out by RNA-Seq. Migration and proliferation studies were performed by holographic microscopy. Cell cycle characterization was performed by flow cytometry. Induction of apoptosis was assessed by determining the expression of apoptosis-related genes using RT-qPCR. MTT assay was performed to determine the effect on cell viability of anti-tumor drugs (cisplatin, oxaliplatin, gemcitabine, 5-FU, sorafenib, and regorafenib).

Results: In silico analysis showed that PCBP2 is abundantly expressed in CCA, which was confirmed experimentally in tumor samples at mRNA and protein levels. Both iCCA and eCCA tissue samples analyzed by immunohistochemistry presented significantly stronger staining of PCBP2 at the nuclei of tumor cells than non-tumor cholangiocytes. Compared with Mock cells, silencing of PCBP2 in liver cancer cell lines did not induce changes in the response to the studied anti-tumor drugs. In contrast, a significant decrease in cell proliferation and viability, a lower migration capacity, and enhanced expression of the pro-apoptotic factor BAX, consistent with apoptosis activation, were found. RNA-Seq analysis revealed that PCBP2 silencing affected the expression of several genes known to be associated with the oncogenic process, i.e., downregulation of MEX3A, NRIP1, and NRP2 and altered the alternative splicing pattern of MAP4K4.

Conclusion: PCBP2 may act as a relevant factor in regulating cell migration and proliferation in CCA and, therefore, it could be considered as a potential target for the development of novel strategies to treat this cancer.

PO1-10-YI

Manipulating tumour associated macrophages to elicit anticancer effects in a spheroid-engrafted precision-cut liver slice model of human hepatocellular carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) is the most prevalent form of primary liver cancer. Immunotherapy is the first-line treatment for advanced HCC, which commonly develops on a background of chronic liver disease resultant of metabolic-associated steatohepatitis (MASH). A paradoxical effect is often observed in patients with MASH whereby immunotherapy promotes tumour progression due to an exhausted subset of CD8+PD-L1+ T cells, demonstrating the complexity of the tumour immune microenvironment (TIME). Macrophages are the most abundant myeloid cell in the liver and are therefore attractive targets in the TIME to elicit anti-cancer activity and enable immunotherapy. We developed a spheroid-engrafted precision-cut liver slice (PCLS) model of human HCC which enables an interactive TIME to be maintained for up to one week in culture. Using this platform we investigated the impact of cenicriviroc (CVC), a CCR2/CCR5 inhibitor previously trialled for the treatment of MASH, on HCC.

Method: Spheroids were generated from patient-derived HCC cells and implanted on human PCLS. Multiphoton (MP) imaging was employed to illustrate that HCC spheroids displayed complete engraftment and invasion in PCLS one day after spheroid implantation. Hyperion imaging mass cytometry (IMC) was performed to provide a spatial characterisation of the engineered TIMEs. To elucidate the impact of CVC on the HCC TIME, human PCLS were treated with or without CVC prior to spheroid implantation and subsequent IMC to determine the impact on myeloid cells.

Results: MP imaging and IMC performed on spheroid-engrafted PCLS illustrated the presence of an interactive TIME, whereby engrafted HCC cells migrate into surrounding liver tissue and immune cells, such as CD163+ macrophages and CD8+ T cells, migrate into the spheroid. This is accompanied by the development of an alpha-SMA+ stroma and a CD31+ endothelium which co-localises with vimentin expression, suggesting *ex vivo* neoangiogenesis. Patient-derived HCC spheroids were shown to secrete levels of CCL2 comparative to their ability to recruit THP-1 cells in an *in vitro* monocyte migration assay. Subsequently, pre-treatment of PCLS with CVC was shown to inhibit the recruitment of immunosuppressive CD163+ cells to the HCC region of spheroid-PCLS, indicating that CVC may elicit favourable anti-cancer effects in a cancer type with an abundance of immunosuppressive myeloid cells.

Conclusion: The engineered HCC spheroid-PCLS model allows TIME activity to be investigated and manipulated in the context of human disease. Preliminary findings indicate that CVC has potential to manipulate the TIME in HCC and its efficacy is currently being examined *in vivo*.

PO1-11-YI
Therapeutic potential of Human antigen R inhibition in chronic liver disease and hepatocellular carcinoma

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Background and aims: The RNA-binding protein HuR (Human antigen R) importantly regulates the stability and translation of a wide range of transcripts involved in metabolism, inflammation, and cancer (e.g., TNFα, MYC). While several studies have highlighted the tumor-promoting function of HuR in hepatocellular carcinoma (HCC), emerging evidence indicates that HuR inhibition may promote Metabolic Dysfunction-Associated Steatohepatitis (MASH). Therefore, the role of HuR in these diseases remains unclear and the therapeutic potential of HuR inhibition in these diseases remains to be demonstrated. In this study, we have characterized the impact of HuR inhibition on both HCC and MASH development.

Method: HuR expression was measured in liver tissues from patients and mouse models of hepatic steatosis, MASH, HCC, and intrahepatic cholangiocarcinoma (ICC). The potential of HuR inhibition was evaluated by genetic (siRNA, AAV8) or pharmacologic approaches (HuR inhibitors: CMLD-2 and MS-444) in a panel of hepatic cancer cell lines, primary hepatocytes and mouse models of MASH (mice fed a choline-deficient amino acid-defined high-fat diet: CDAHFD). A proteomic analysis in hepatic cancer cells was performed to characterize the impact of HuR inhibition on cancer-related processes and identify potential targets.

Results: : HuR is overexpressed in HCC and ICC as compared to non-tumoral tissues. Histological analyses revealed that some patients exhibit a strict nuclear localization pattern, while others display nuclear and cytoplasmic localization. HuR inhibitors markedly reduced hepatic cancer cell proliferation, viability and migration. For MS-444, but not for CMLD-2 or HuR silencing, cells accumulated in prophase, thus suggesting some HuR-independent effects. A proteomic analysis confirmed that HuR inhibitors alter a network of oncogenes and tumor suppressors, including known HuR targets (e.g., ATG5), associated with a poor prognosis. However, HuR inhibition promotes hepatic steatosis in primary hepatocytes and in mice fed a CDAHFD, through the downregulation of ApoB.

Conclusion: HuR is an important tumor promoter in HCC and HuR inhibitors display interesting anti-cancerous properties in hepatic cancer cells. However, HuR inhibition also exacerbates hepatic lipid metabolism and thus promotes MASH development, thus suggesting cautions regarding the use of HuR inhibitors in clinical practice.

PO1-12-YI

Scavenger receptor MARCO is associated with an immunosuppressive microenvironment and tumor progression in intrahepatic cholangiocarcinoma

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Background and aims: Cholangiocarcinoma (CCA) comprises a heterogeneous group of malignant tumors with dismal prognosis. The immune system plays a key role in the development of intrahepatic CCA (iCCA) and several combinational therapies targeting the tumor microenvironment (TME) have shown promising results for anti-cancer therapy. The macrophage receptor with collagenous structure (MARCO) is a class A scavenger receptor found on particular subsets of macrophages that plays a determining role in macrophage polarization and consequently in adaptive immune responses in many solid tumors. This study aims to unravel the role of MARCO in iCCA.

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

Results: Single-cell RNA sequencing data indicate that *MARCO* is expressed in a specific subtype of TAMs associated to processes of immunosuppression and extracellular matrix (ECM) remodeling in patients with iCCA. Besides, high *MARCO* expression levels in iCCA tumours are linked with worse clinical outcomes and T cell dysfunction. In murine models of iCCA, in a context of a syngeneic orthotopic experimental model, *Marco*$^{-/-}$ mice exhibit a reduced presence of immune checkpoint molecules in innate and adaptive immune cells, including a lower percentage of PD-L1+Ly6C-F4/80+ macrophages and, PD-1+ and CTLA-4+ cytotoxic CD8+ T cells in comparison to WT mice. MARCO deficiency significantly improves the overall survival of mice subjected to this experimental model, and this was associated to a reduction of the tumor metastasis in the lungs. Notably, employing an anti-MARCO antibody further reduced tumor volume in wild-type mice. In addition, *Marco*$^{-/-}$ mice are partially protected from iCCA development in two independent experimental models, associated to a reduction

of the innate immune cells such as CD9+Ly6C-F4/80+ macrophages and type-2 innate lymphoid cells (ILC2) that promote a T_H2 response.

Conclusion: High MARCO expression levels are associated with worse overall survival and an immunosuppressive TME. Therefore, MARCO arises as a novel prognostic and therapeutic target in iCCA.

PO1-13-YI
Molecular characterization of hemochromatosis tumors and iron overload in hepatocellular carcinoma

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Background and aims: Hemochromatosis (HM) is a genetic disease characterized by systemic iron overload. Traditionally, diagnosis of HM relies on the homozygosity of *HFE* C282Y in European patients. These patients are at increased risk of developing liver diseases and hepatocellular carcinoma (HCC). We hypothesized that the genetics of HM might influence the mechanisms of carcinogenesis in the liver and aimed to characterize the molecular features of HM related HCCs.

Method: First, we performed genotyping analysis in a cohort of 1,793 HCC cases and 2,166 controls without cancer to explore the risk of HCC associated with frequent variants in HM genes (*HFE*, *HAMP*, *HJV*, *SLC40A1, TFR2*). Variant frequencies were also tested against the general population. Next, rare variants and mutations were identified in 355 HCC patients of European origin using WES/WGS. Data was validated in 115 HCC patients of non-European ancestry. Molecular features of HM HCCs and iron overload were evaluated in patients with non- viral etiologies.

Results: Analysis of frequent variants in genotyping data identified homozygous *HFE* C282Y to be associated with higher risk of HCC, compared to controls, and the French general population. This variant was related to lower fibrosis and male gender. No other frequent variants in genes related to HM were found to be associated with HCC in the genotyping cohort. Next, we focused on a cohort of European HCC patients that underwent WES/WGS. In this cohort, we identified rare variants of different pathogenicity and we could validate the risk of HCC related to homozygous *HFE* C282Y. Clinical and molecular features of tumors from HM (16%) HCC patients were compared to those from alcoholic liver disease (46%), metabolic syndrome (35%) and unknown etiology (23%) patients. HM patients were characterized by increased proportion of male gender, smaller tumors and iron accumulation in non-tumor liver tissues. HM tumors showed different distributions of transcriptomic subgroups G1-G6, they were enriched in Hoshida S1 classes, and they displayed more frequent *ALB* mutations (38%). Overall, iron overload was related to more frequent *CTNNB1*, *TSC1* and *ALK* somatic mutations.

Conclusion: Genetics of hemochromatosis and iron overload lead to specific molecular features in hepatocellular carcinoma

PO1-14-YI
Therapeutic Targeting of Neddylation in Hepatoblastoma: Novel Approaches in Pediatric Liver Cancer

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Background and aims: Hepatoblastoma (HB) is a rare type of primary liver cancer that mainly affects children. Currently, the main treatment options include surgical resection accompanied by chemotherapeutics such as cisplatin or doxorubicin. However, these options are limited or ineffective due to poor prognosis, high recurrence rates, and significant side effects. HB is characterized by a low rate of somatic mutations, suggesting the involvement of other mechanisms, as post-translational modifications, that modulate its tumorigenic capacity. Neddylation, in particular, has been extensively studied for its role in cancer biology. Based on this evidence, we hypothesized that neddylation plays a significant role in HB development and progression. Our aim was to analyze the impact of neddylation in HB progression and to explore whether targeting this pathway could offer a new therapeutic approach.

Method: We characterized the Neddylation pathway in HB using a combination of patient samples, an HB preclinical mouse model (YAPS127A/N90-β-catenin), and in vitro tumor cell models (HepT1, HepG2, and patient-derived xenografts (PDX) from distal metastasis). Transcriptomic analysis was performed on samples from HB patients to assess neddylation cycle activity. We examined levels of NEDD8 and NAE1 (NEDD8-activating enzyme E1) and evaluated NEDP1 deneddylase activity. In vitro studies involved modulation of NEDP1 levels to observe effects on cell proliferation, migration, and metabolic status. In vivo, the effects of NEDP1 overexpression were tested for tumor suppression in CAM assays (both in Ovo and ex Ovo) and in the HB mouse model.

Results: Transcriptomic analysis revealed increased neddylation and reduced NEDP1 deneddylase activity in HB patients, tumor cells, and mice models, indicating neddylation's importance in HB progression. Modulating neddylation through NEDP1 overexpression in vitro resulted in apoptosis induction, reduced proliferative and migratory capacities, and metabolic alterations. In vivo, NEDP1 overexpression led to reduced angiogenesis, tumor formation, and metastatic potential in CAM assays. The HB mouse model exhibited significant tumor suppression, characterized by enhanced apoptosis, and proteomic reorganization, suggesting that NEDP1 functions as a tumor suppressor in HB.

Conclusion: Our findings highlight the critical role of neddylation in HB progression and suggest that dysregulation of this pathway, particularly through reduced NEDP1 activity, contributes to tumor growth and metastatic behavior. NEDP1 overexpression demonstrated promising anti-tumor effects, including apoptosis induction, reduced angiogenesis, and decreased tumorigenic and metastatic potential. These results underscore the potential of targeting neddylation, specifically through NEDP1 modulation, as a novel therapeutic strategy for HB.

PO1-15-YI

Exploring the impact of mitochondrial biomarkers on HCC risk according to etiologic drivers: a multicenter study

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Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) overcame both viral (V) and alcohol (ALD) hepatitis, becoming the main cause of hepatocellular carcinoma (HCC). We recently showed that two new circulating mitochondrial (mt-) biomarkers (*D-loop*, ccf-COXIII) increase in MASLD patients. *D-loop* progressively raised with histological damage, resulting highly informative as early MASLD prognostic indicator. Instead, ccf-COXIII is closely related to advanced MASLD forms (cirrhosis, HCC). Still, a combination of both biomarkers accurately predicted HCC risk (86%). However, whether these biomarkers are MASLD-specific remains to be further elucidated. We aimed to assess *D-loop* and ccf-COXIII levels across different etiologies (V, ALD, and MASLD) in a multicenter cohort of 260 HCC patients from Milan, Rome, Verona, and Udine.

Method: *D-loop* and ccf-mtDNA were measured in PBMCs and serum samples, respectively.

Results: MASLD-HCC subjects had higher BMI, presence of diabetes mellitus (T2DM) and serum lipids compared to ALD-HCCs and V-HCCs ($p<0.05$). The highest levels of transaminases and alpha-fetoprotein were found in V-HCC patients. The mean size of HCC nodules was larger in both MASLD-HCCs and V-HCCs rather than ALD-HCCs. ccf-COXIII levels were higher in V-HCC patients compared to non-viral HCCs ($p<0.001$) at bivariate analysis and the association with viral etiology was statistically significant at generalized linear model adjusted for sex, age at diagnosis, BMI, T2DM, and ALD/MASLD etiologies. At nominal logistic analysis adjusted as above, the ccf-COXIII correlated with increased risk of developing HCC from viral infections (OR:1.27 95% CI:1.08-1.5, $p=0.0009$). However, this association was lost after the correction for cirrhosis. Unlike, the highest levels of circulating *D-loop* were observed in MASLD-HCC patients and correlated with tumor size. Moreover, the association between circulating D-loop and MASLD etiology was confirmed at multivariate analysis. Alongside, elevated *D-loop* levels correlated with enhanced cancer risk of metabolic origin (OR:1.62 95% CI:1.0-2.5, $p=0.02$), even after adjustment for the presence of cirrhosis.

Conclusion: Mt-biomarkers change in HCC patients according to etiology. ccf-COXIII rises most with virus infections in advanced liver disease, while *D-loop* seems to be MASLD-specific, possibly identifying HCC cases also in the absence of cirrhosis.

PO1-16
Lipid and cell cycling perturbations driven by the HDAC inhibitor romidepsin render liver cancer vulnerable to receptor tyrosine kinase targeting and immunologically active

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Background and aims: Despite the revolution of immunotherapies for Hepatocellular Carcinoma (HCC) treatment, the receptor tyrosine kinase inhibitors (RTKi) remain the lifejacket for a high proportion of HCC patients. Hence, research on new strategies to enhance RTKi's efficiency is still up to date. In this work we explored the therapeutic effects of romidpensin, a class I HDAC inhibitor (HDACi), alone and in combination with RTKi currently used in the clinic for HCC treatment.

Method: We analysed different HCC patient cohorts (transcriptomics and proteomics), to explore HDACs expression and their correlation with overall survival. We selected a panel of human HCC cell lines that are resistant to RTKi to test the efficiency of a combined treatment of RTKi with HDACi. We performed proteomic, lipidomic, biochemical and molecular analysis to understand the mechanisms of action of this combination. We have corroborated the efficiency of this combination on patient-derived tumoroids and different HCC mouse models followed by microphoton-counting computed-tomography and bioluminescence, and by analysing the immune cell composition and the expression of several immune-checkpoints in the tumoral microenvironment by spectral cytometry.

Results: We found that HDAC1/2 are overexpressed in HCC patients, and correlate to worse prognosis, unlike other class I HDACs. We show how romidepsin affects the levels of different cell cycle and survival signals, and confers sensitivity to RTKi in a panel of human HCC cells otherwise resistant. Mechanistically, romidepsin affects the mitotic spindle machinery, which leads to the formation of a monopolar mitotic spindle, alters chromosome segregation and blocks cells into mitosis. Furthermore, romidepsin modifies the expression of several lipid metabolism regulators, remodelling specific lipid species, through LXR/RXR -dependent (Srebf1 upregulation) and -independent pathways. The combined treatment with romidepsin and RTKi Cabozantinib (RomiCabo) in HCC cells switches the cytostatic effect of romidepsin alone into apoptotic cell death. The therapeutic effect of RomiCabo is also demonstrated in HCC patient-derived tumoroids and in several HCC mouse models that recapitulate resistance and heterogeneity found in HCC patients. Moreover, we document how RomiCabo leads to a remodelling of the immune profile in the tumour microenvironment, conferring an immunostimulatory profile.

Conclusion: Overall, our results demonstrate that romidepsin sensitizes HCC to RTKi, through a combinatorial action at epigenetic, metabolic, and immunological levels. Moreover, our in vivo outcomes illustrating a striking remodelling of the immune cell types and immune-checkpoint after RomiCabo treatment may be exploited to optimize immunotherapy for HCC.

PO1-17
ATR, guardian of genome integrity, a new player to optimize high replication stress hepatocarcinoma therapy

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Background and aims: Hepatocellular carcinoma (HCC) belongs to the five most common malignancies in adult. Targeting DNA Damage Response (DDR) components has emerged as a promising approach for cancer therapy. In this study, we investigated the potential therapeutic benefits of targeting the ATR kinase in HCC.

Methods: A cohort of HCC patients was analyzed from the TCGA (N=307) and LICA-FR (N=222) datasets. Patients were categorized into two groups based on high and low replication stress (RS) signatures. Pre-clinical murine HCC models (non-germline mosaic GEMMs) were employed. Liver-specific deletion of ATR was achieved in ATRDhep mice via AAV8-CRISPR/Cas9, with ROSA26Dhep mice serving as controls. Cellular and molecular analyses were performed to assess cell proliferation. Flow cytometry and immunostaining were used to profile immune cells. RNA-Seq was conducted to investigate the impact of ATR inhibition on high RS murine HCC.

Results: A gene transcriptomic signature associated with DNA maintenance, replication, and cell cycle regulation was used to assess the prognostic significance of RS in HCC. In two independent cohorts, we demonstrated that patients with a high RS signature exhibited significantly worse progression-free and disease-specific survival compared to those with a low RS signature. Our data further revealed that RS is enriched in HCC subtypes harboring TP53 and CCNA2/E1 mutations, but not CTNNB1 mutations. To model human liver tumors with low or high RS signatures, we established preclinical mouse models of HCC. We demonstrated that silencing ATR significantly reduced tumor growth in a high RS model (MYC overexpression/p53 loss) but had no effect on tumor growth in a low RS model (MYC overexpression/CTNNB1 activation). Further investigation showed that ATR inhibition in MYC; sg-p53 livers improved survival. Mechanistically, this effect appears to be mediated by suppressing hepatocyte proliferation and reshaping the immune microenvironment towards an anti-tumor response, particularly through increased CD8+ T cell infiltration.

Conclusion: Our findings suggest that the RS signature can identify a subgroup of HCC patients who may benefit from ATR-based therapies.

PO1-18
The usefulness of novel cholangiocarcinoma cell lines with enhanced resistance to cisplatin or 5-fluorouracil to study mechanisms of chemoresistance

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Background and aims: Pharmacological treatment of cholangiocarcinoma (CCA) is often hampered by tumor resistance to chemotherapy, primarily due to active mechanisms of chemoresistance (MOCs). Advancing our understanding of resistome is essential to developing strategies able to overcome drug refractoriness in CCA. The establishment of novel human cell lines resistant to chemotherapy could be instrumental in identifying novel therapeutic targets to overcome CCA chemoresistance.

Method: To develop and characterize CCA-derived 5-fluorouracil (5-FU)- and cisplatin-resistant cell sublines, cells previously established from extrahepatic (EGI-1) and intrahepatic (HuCCT1) CCA were exposed to stepwise increasing concentrations of cisplatin (up to 9 µM) or 5-FU (up to 10 µM). Cross-resistance to other commonly used antitumor drugs was determined in resistant cells using the MTT test. Migration and proliferation were analysed using holographic microscopy. Taqman Low-Density Arrays (TLDAs) were used to quantify the mRNA expression of ~100 genes involved in the resistome. Additionally, RT-qPCR, western blotting, and immunofluorescence were used to evaluate gene and protein alterations potentially contributing to drug resistance.

Results: The drug-resistant cell sublines EGI1/CR and HuCCT1/CR (cisplatin-resistant) and EGI1/FR (5-FU-resistant) were successfully established. These cells maintained their drug-resistant phenotype in drug-free medium for at least eight passages. MTT assays confirmed that EGI1/CR and EGI/FR exhibited over 6-fold increased resistance to cisplatin and 5-FU, respectively, compared to parental cells, while HuCCT1/CR cells showed a 2-fold increase in cisplatin resistance. Cisplatin-resistant cells (both EGI1/CR and HuCCT1/CR) were cross-resistant to oxaliplatin. EGI1/FR cells exhibited cross-resistant to gemcitabine and irinotecan, suggesting a multidrug-resistant phenotype. Interestingly, all resistant cells showed reduced proliferation and migration rates compared to their parental counterparts. Regarding multidrug resistance, these cells showed upregulation of specific multidrug resistance proteins (MRPs), with MRP3 and MRP6 upregulated in cisplatin-resistant cell lines and MRP4 in EGI1/FR cells, contributing to increased drug efflux. Other remarkable changes included alterations of drug metabolism, with upregulation of UGT1A in EGI1/CR and UPP1 in HuCCT1/CR cells. In EGI1/CR cells a shift toward enhanced expression of cancer stem cell-associated genes, such as SOX2, was found.

Conclusion: Three different cisplatin or 5-FU-resistant CCA cell sublines have been obtained and characterized. Analysis of the resistome revealed significant changes, including the upregulation of ABC pumps involved in drug efflux, as well as alteration in genes involved in drug metabolism and cancer cell stemness.

PO1-19

Antitumoral activity of G9a inhibitors in hepatocellular carcinoma and its potential combination with immune checkpoint inhibitors

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Background and aims: Treatment of advanced Hepatocellular Carcinoma (HCC) has significantly improved due to the advent of immune-checkpoint inhibitors (ICIs). However, many patients still show innate or acquired resistance to ICI-based therapies. Hence, new therapeutic strategies to combat resistance and enhance effectiveness are needed. Epigenetic alterations play a crucial role in liver cancer development. These alterations can suppress the production of certain proteins that aid in immune response against tumors such as chemokines and/or the antigen presentation machinery. Our research has identified a promising target for liver cancer treatment, the histone methyltransferase G9a. We conducted experiments to evaluate the effectiveness of specific inhibitors of G9a (EZM8266 and CM272), both in vitro and in vivo and their potential combination with ICIs.

Method: RNAseq analyses of murine HCC cells treated with CM272 alone or combined with IFNɣ were performed. Validation of the data was conducted by using a second G9a inhibitor, EZM8266 and specific siRNAs. Cytokine release was measured by ELISA. MHC was evaluated by flow cytometry analysis. The effects of both G9a inhibitors, CM272 and EZM8266, on the growth of PM299L cells orthotopically implanted in immunocompetent mice, alone and in combination with α- PD1 antibodies, were also determined. Multiplex immunofluorescence of tissues was performed to evaluate changes in immune cell populations.

Results: Inhibition of G9a, either genetically or pharmacologically in HCC cells led to a significant stimulation of immune response-related genes. The release of chemokines CXCL9 and CXCL10 was validated by ELISA, and increased MHC-Class I membrane exposure was confirmed by cytometric analysis following G9a inhibition in HCC cells. Mechanistically, transcriptomic analysis showed a substantial upregulation of genes reported as a primary regulators of MHC presentation-related molecules. These results were confirmed through real-time PCR in all tested cell lines. In vivo, both G9a inhibitors inhibited the growth of orthotopically implanted tumors and enhanced the effects of α-PD1, resulting in remarkable antitumor activity. These responses were accompanied by increased CD8, CD4, CD11b, and FOXP3 positive cells infiltration. No signs of systemic or liver toxicity were observed.

Conclusion: G9a is confirmed as an effective druggable target in HCC. Pharmacological inhibition of G9a increases the expression of immune response-related genes and enhances the efficacy of ICIs. Our findings provide strong support for the combination immunotherapy with epigenetic drugs such as CM272 and EZM8266 for HCC treatment.

PO1-20
Proteomic profiling of advanced hepatocellular carcinoma identifies predictive signatures of response to treatments

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Background and aims: Hepatocellular carcinoma (HCC) is the most common form of liver cancer but, due to late diagnosis, the prognosis is extremely poor and the majority of cases are advanced HCC, which are only eligible for palliative systemic therapies. After a decade of exclusive sorafenib monotherapy, with a response rate of < 10%, the advent of new combinations including immunotherapies represents a revolution in the management of HCC. The combination of atezolizumab/bevacizumab is recommended as the first-line systemic treatment for HCC, with a response rate of no more than 30%. Additionally, less than 25% of non-responding patients are eligible for second-line treatment. However, there are currently no predictive factors for response to these different treatment options.

Method: We profiled, by high-resolution mass spectrometry-based proteomics combined with machine learning analysis, a selected cohort of formalin-fixed, paraffin-embedded biopsies of advanced HCC adenomas with clinical follow-up collected from several French hospitals. We grouped subject according to their objective response to treatments, corresponded to a tumor regression > 30% (G1) and tumor progression > 20% (G2) at 6 months after the beginning for atezolizumab/bevacizumab and sorafenib treatments.

Results: We generated a proteome database of 50 selected HCC samples. We compared the relative protein abundance between tumoral and non-tumoral liver tissues from advanced HCC patients treated with atezolizumab/bevacizumab or sorafenib. The clear distinction of these two groups for each treatment using principal component analysis with a deregulation for 141 protein or 87 for atezolizumab /bevacizumab and sorafenib treatment, respectively. These signatures were sufficient to predict the response. Moreover, these signatures reveal the biological pathways involved in the resistance to treatments. Particularly, we revealed and validated a shift in tumor cell energy metabolism inducing an immunosuppressive environment involved in the resistance to atezolizumab/bevacizumab.

Conclusion: We performed an in-depth analysis of quantitative proteomic data from HCC biopsies using statistical packages and found that their proteome includes information to predict the treatment response to advanced HCC giving the ability to optimize patient management.

PO1-21
Analysis of intrahepatic cccDNA and HBV-DNA in patients with hepatocellular carcinoma attributed to non-HBV etiologies

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Background and aims: Hepatitis B virus (HBV) is an oncogenic virus. Previous studies have linked serum anti-HBc and HBV-DNA in HBsAg-negative patients to increased hepatocellular carcinoma (HCC) risk, particularly among Asian patients. This study aimed to evaluate the presence of intrahepatic covalently closed circular DNA (cccDNA) and HBV-DNA in a cohort of HCC patients and its relationship to serum HBV markers.

Method: Prospective cohort of 64 HBsAg-negative HCC patients from whom paired tumor tissue, adjacent non-tumor tissue, and serum samples were collected between January 2016 and January 2024. cccDNA and intrahepatic HBV-DNA (iHBV-DNA) in both tumor and adjacent tissues were analyzed and quantified using qPCR. Serum anti-HBc antibodies and serum HBV DNA were determined using commercial assays (Roche Diagnostics). 84% were males with a median age of 62 years, 91% Caucasian, and 96% had underlying liver disease (HCV [36%], ALD [17%], and MASLD [16%]). Additionally, six HBsAg+ patient were analyzed.

Results: Of the 64 HBsAg-negative HCC patients, 42% (27/64) exhibited iHBV-DNA in either tumor or adjacent tissue [mean (SD): 0.005 (0.008) copies/cell], and 6% (4/64) had detectable cccDNA [0.004 (0.004) copies/cell].
Serum anti-HBc was found in 19 (30%) patients, with iHBV-DNA also detected in tumor or adjacent tissue in 5 (26%) of these cases. Among anti-HBc-negative patients, 49% (22/45) had iHBV-DNA in tissue, with cccDNA present in 4 cases. No significant difference was noted in iHBV-DNA presence or concentration based on anti-HBc status. None of the four patients with cccDNA had HBV DNA in serum. All 6 HBsAg-positive (HBeAg-negative) patients had iHBV-DNA in both tumor and adjacent tissues [11.74 (11.51) and 3.57 (3.89) copies/cell, respectively], with cccDNA detected in 100% of adjacent tissues [0.59 (0.61)] and 50% of tumor tissues [0.56 (1.22)]. The iHBV-DNA concentration in HBsAg-positive patients was significantly higher than in HBsAg-negative patients in both tumor [11.74 (15.37) vs. 0.008 (0.011)] and adjacent tissues [3.57 (3.89) vs. 0.0023 (0.0018), $p<0.001$].

Among the 27 HBsAg-negative patients with iHBV-DNA and/or cccDNA in tissue, underlying liver disease was attributed to HCV (37%), MASLD (33.3%), or other non-HBV-related causes, with no significant differences in iHBV-DNA concentration by etiology.

Conclusion: Nearly half of HBsAg-negative HCC patients attributed to non-HBV etiologies show intrahepatic HBV DNA or cccDNA. Detection is not related to serum anti-HBc, suggesting high HBV exposure. Further molecular analyses are ongoing.

PO1-22
Shaping the immune microenvironment of hepatocellular carcinoma tumours: a role for senescent endothelial cells

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Background and aims: Senescence is known to significantly contribute to a range of solid cancers, including hepatocellular carcinoma (HCC). Senescent cells release a complex inflammatory cocktail, termed the "senescence-associated secretory phenotype" (SASP), and we have previously shown that liver-derived endothelial cells can mediate immune cell recruitment when acutely exposed to SASP in both *in vitro* and *in vivo* models. Prolonged exposure to SASP can also induce paracrine senescence in neighbouring cells; but, to our knowledge, paracrine senescence within HCC tumour endothelial cells has not been explored previously and its impact on the immune microenvironment of HCC tumours is unknown.

Method: We conducted RNA sequencing of endothelial cells isolated from HCC tumours and matched non-tumour tissues, and explored the expression of a published endothelial-specific senescent gene signature, EC.SENESCENCE.SIG. In addition, we performed immunofluorescent staining to identify senescent endothelial cells in human HCC tumour tissues. Next, to recapitulate paracrine senescence of HCC tumour endothelial cells *in vitro*, we derived SASP from ER:RasG12V IMR90 cells (Ras) and control supernatants from growing IMR90 cells (Grow), and challenged isolated primary liver endothelial cells for 7 days. Following this, we confirmed paracrine senescence and undertook flow-based adhesion assays to study the recruitment of isolated $CD4^+$ and $CD8^+$ T cells under physiological flow conditions *in vitro*.

Results: Here, we demonstrate that isolated HCC tumour endothelial cells exhibit a strong upregulation of the senescent gene signature. Additionally, $CD34^+$ tumour endothelial cells in HCC tissues exhibit high expression of the senescence marker, p16. Next, we confirm that isolated primary liver endothelial cells treated with SASP (Ras) for 7 days undergo paracrine senescence, with increased cell and nuclear size, and a higher incidence of multinucleation and SA-β-gal staining. Finally, we demonstrate that liver endothelial cells undergoing paracrine senescence are able to support the adhesion and transendothelial migration of $CD4^+$ and $CD8^+$ T cells under physiological flow, when compared to non-senescent control cells.

Conclusion: We demonstrate that isolated HCC tumour endothelial cells exhibit a strong senescent gene signature and confirm the presence of senescent endothelial cells within human HCC tumour tissues *ex vivo*. We further show that primary liver endothelial cells undergoing paracrine senescence can mediate the recruitment of $CD4^+$ and $CD8^+$ T lymphocytes *in vitro*. Our data suggests that tumour endothelial cells undergoing paracrine senescence have the capacity to shape the immune microenvironment of HCC tumours; however, further characterisation of the specific immune cell subsets recruited is required.

PO1-23
Spatial transcriptomics characterization of liver microenvironment in MASH and MetALD HCCs

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Background and aims: MASH accounts for 20% of HCCs in the Western world and is HCC leading cause in patients without cirrhosis. The higher propensity for HCC development without cirrhosis in MASH compared with other etiologies underscores the unique metabolic and inflammatory micro-environment that cooperate with extrahepatic cancer drivers associated with metabolic syndrome and, when present, alcohol consumption. Here we investigate the metabolic and inflammatory microenvironment of MASH HCC using Digital Spatial Profiling (DSP) technology in multiple tumour (T) and non tumour (NT) regions of interest (ROIs) from surgical resection samples of cirrhotic and non cirrhotic patients with HCC related to MASH and MetALD.

Method: The study population comprised 5 cirrhotic (K) and 4 non cirrhotic (F1/F2/F2/F3) SLD HCC patients. Six had no alcohol consumption (F1/F2/K/K/K) and 3 were MASLD predominant Met-ALD (F2/F3/K). Spatial resolution of RNA expression from >1,800 genes was assessed using the GeoMx® Cancer Transcriptome Atlas in T and NT ROIs of FFPE (Formalin-Fixed Paraffin-Embedded) tissue samples. A masking approach was used to define homogeneous areas of interest (AOI) containing PanCK or CD45 labelled cells in each ROI. All cells present in each AOI in T or NT livers were analyzed separately as a mini-bulk RNA-Seq.

Results: 95 ROIs (range 9-17 ROIs per sample) have been analyzed. After QC 82 ROIs were retained for a total number of 38938 PanCK positive cells (hepatocytes, cholangiocytes and tumor cells) and of 4239 CD45 positive cells. Principal Component Analysis of all ROIs showed a clear separation between PanCK AOIs and CD45 AOIs in T vs NT. Focusing on CD45 ROIs, we found that the inflammatory infiltrate, richer in NT liver, displays distinct transcriptional profiles in T vs NT; with 13 down-regulated and 26 up-regulated differentially expressed genes. CD45 AOIs enrich oxidative phosphorylation and integrins signaling pathways and downregulate lymphocyte effector pathways in T tissues. WGCNA (weighted gene co-expression network analysis) of all CD45 ROIs identified 5 modules of highly connected and co-expressed genes (hub genes) associated with tumor status. Additional modules were specific for CD45 AOIs stratified according to the presence of cirrhosis, MASH or Met-ALD. Cell type deconvolution analysis of CD45 and PanCK AOIs stratified according to tumor status show an enrichment of Tregs, central venous LSECs and stellate cells in tumor vs non-tumor tissue.

Conclusion: Our DSP analysis allowed a highly granular spatial resolution of the transcriptome and the microenvironment in specific subgroups of MASH HCCs.

PO2-01
The Role of FOXO1 in Post-Transplant Recurrence of Hepatocellular Carcinoma: A Study on Diagnostic and Therapeutic Targets

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Background and aims: Hepatocellular carcinoma (HCC) is a highly prevalent and deadly malignancy in China. Although liver transplantation is the most effective treatment for HCC, tumor recurrence remains a significant challenge. Identifying precise diagnostic and therapeutic targets for post-transplant recurrence is crucial for improving HCC outcomes. This study aims to investigate the clinical association between FOXO1 and liver transplant outcomes in HCC patients and to explore the therapeutic potential of an esterase-responsive cationic liposome-coated nanocomplex targeting the liver in animal models.

Method: We analyzed a tissue microarray of liver transplant samples from HCC patients (n = 259) to elucidate the correlation between FOXO1 expression and clinical parameters. We then constructed an esterase-responsive cationic liposome-coated nanocomplex carrying FOXO1, targeting the liver, and evaluated its effects in vivo on tumor recurrence post-transplant.

Results: Analysis of the liver transplant tissue microarray revealed that recipients with low FOXO1 expression had significantly shorter tumor-free survival (P = 0.010) and overall survival (P = 0.019) compared to those with high FOXO1 expression. Animal experiments showed that hepatic ischemia-reperfusion injury (IRI) induced changes in key inflammatory (TNF-α and IL-6) and oxidative stress proteins (Nrf-2 and HO-1), promoting tumor growth. Treatment with the liver-targeted FOXO1 esterase-responsive cationic liposome-coated nanocomplex significantly reduced tumor size in mice post-IRI compared to controls. Additionally, alanine aminotransferase and aspartate aminotransferase levels and liver histology (HE staining) indicated reduced IRI in treated mice. The nanocomplex treatment also decreased inflammatory protein expression and increased oxidative stress protein expression in the liver.

Conclusion: Low FOXO1 expression is a risk factor for post-transplant recurrence of HCC. FOXO1 can inhibit HCC progression by mitigating oxidative stress and inflammatory responses induced by hepatic IRI, providing a new strategy for the diagnosis and treatment of tumor recurrence after liver transplantation in HCC patients.

PO2-02
Molecular Phenotypic Linkage Between N6-methyladenosine Methylation and Tumor Immune Microenvironment in Hepatocellular Carcinoma

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Background and aims: The crucial role of N^6-methyladenosine (m^6A) methylation in anti-tumor immunity and immunotherapy has been broadly depicted. However, the molecular phenotypic linkages between m^6A modification pattern and immunological ecosystem are expected to be disentangled in hepatocellular carcinoma (HCC), for immunotherapeutic unresponsiveness circumvention and combination with promising drug agents.

Method: Modification patterns of m^6A methylation were qualitatively dissected according to the large-scale HCC samples profiling. We then determined the immune phenotypic linkages by systematically evaluating their tumor microenvironment composition, immune/stromal-relevant signature, immune checkpoints correlation and prognostic value. Individual quantification of m^6A methylation pattern was achieved by m^6Ascore construction, intensified by longitudinal single-cell analysis of immunotherapy cohort and validated by the transcriptomic profiles of our in-hospital GDPH-HCC cohort. Candidate therapeutic agents were also screened out.

Results: Three distinct m^6A methylation patterns were determined in high accordance with inflamed-, excluded- and desert- immunophenotype. To be precise, Immune-inflamed high-m^6Ascore group was characterized by activated immunity with favorable prognosis. Stromal activation and absence of immune cell infiltration were observed in low-m^6Ascore phenotype, linked to impaired outcome. Patients with low-m^6Ascore demonstrated diminished responses and clinical benefits for cohorts receiving immunotherapy. The above credible linkage between m^6A methylation pattern and tumor immune microenvironment was robustly validated in our GDPH-HCC cohort. Single-cell dynamic change of m^6A methylation level in exhausted CD8 T cell and fibroblast was depicted in immunotherapy cohort fore and art. Derived from m^6A methylation pattern, seven potential frontline drug agents were recognized as promising choice for high-m^6Ascore patients.

Conclusion: Our work bridged the credible linkage between epigenetics and anti-tumor immunity in HCC, unraveling m6A modification pattern as immunological indicator and predictor for immunotherapy. Individualized m6Ascore facilitated strategic choices to maximize therapy-responsive possibility.

PO2-03-YI
Harnessing human immune system mouse models to validate NOX1 inhibition as a treatment for hepatocellular carcinoma

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Background and aims: NADPH oxidases (NOX) are reactive oxygen-producing enzymes of which the isoform NOX1 is present in the tumor microenvironment (TME) of hepatocellular carcinoma (HCC) and promotes HCC development and metastasis. NOX1 inhibition (NOX1i) has been shown to alter the TME in classical HCC models and might serve as a therapeutic target. However, implementation in the clinic is hampered by low translatability of these classical HCC models. Here, we employed next-generation human immune system (HIS)-HCC models that enable investigation of treatments in the context of human immune responses, and provide evidence for NOX1 inhibition as strategy to target the TME in HCC.

Method: For the myeloid (My-)HIS-HCC model with focus on monocytes/macrophages, irradiated NSG-QUAD mice were reconstituted with human cord blood-derived CD34+ cells, and human orthotopic HCC tumors were induced 4 weeks post engraftment. For the T-cell-HIS model, orthotopic HCC tumors were induced in NSG mice, and mice were reconstituted with human T cells using healthy peripheral blood mononuclear cells. Mice received NOX1i (ML171) or vehicle twice per week for 3 weeks.

Results: In the My-HIS-HCC model, humanization in livers and tumors reached 30-50% and consisted of monocytes, macrophages, dendritic cells, NK cells and B cells. Significantly lower human CD45+ cells were observed in tumor and surrounding tissue of NOX1i-treated My-HIS-HCC mice. NOX1i did not affect macroscopic tumor growth in My-HIS-HCC mice. However, the expression of HCC and proliferation markers was lower in tumors of NOX1i-treated My-HIS-HCC mice. This was accompanied with significantly lower expression of cytokines, chemokines, growth factors and markers involved in cancer progression upon NOX1i. Humanization in the T cell-HIS-HCC model reached up to 70% and primarily consisted of T cells, with significantly reduced percentages in livers of NOX1i-treated mice. Within the population of CD4+ T cells, mainly Th2 cells were encountered without differences between groups. NOX1i did not affect tumor size nor the expression of HCC and proliferation markers. However, as in the My-HIS-HCC model, the expression of cytokines and markers involved in cancer progression was significantly lower in tumor tissue of NOX1i-treated, compared to untreated, T cell-HIS-HCC mice.

Conclusion: Our data show that NOX1i effectively alters the TME in humanized HCC models, with the most pronounced effects observed in mice harboring human monocytes and macrophages, the My-HIS-HCC model. This aligns with previous reports on the therapeutic potential of NOX1i in classical HCC models by affecting monocyte/macrophage function, and favors translation to the clinic.

PO2-04-YI
Deciphering the Glycosylation Status of Programmed Cell Death Ligand 1 and its regulation by Aurora Kinase A in Hepatocellular Carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) remains a major public health issue, ranking as the fourth leading cause of cancer-related deaths worldwide. Programmed Cell Death Ligand 1 (PD-L1) is an immune checkpoint molecule targeted in HCC, yet only a subset of patients responds positively to PD-L1 inhibitors. This limited response suggests complex regulatory mechanisms that control PD-L1 within HCC's heterogeneous tumor microenvironment. PD-L1 undergoes extensive N-linked glycosylation, a post-translational modification that influences protein stability, expression, and response to modulation by upstream effectors. Aurora Kinase A (AURKA), a promising therapeutic target in HCC, has shown potential in regulating PD-L1, though conflicting evidence leaves the precise nature of this modulation unclear. This study aims to determine the glycosylation status and stability of PD-L1 in HCC, assess the expression of PD-L1 following AURKA inhibition, and assess the potential regulatory feedback loop between AURKA and PD-L1.

Method: The glycosylation status of PD-L1 was examined using a PNGase F glycan cleavage assay. PD-L1 stability was determined using cycloheximide (a protein synthesis inhibitor) and MG-132 (a proteasome inhibitor). To inhibit AURKA, HCC cells were treated with the selective AURKA inhibitor, AK-01, and siRNA-AURKA. PD-L1 silencing was also performed to evaluate its effect on AURKA expression. JHH6 and HuH7 cell lines served as *in vitro* models of HCC. PD-L1 and AURKA expression were analyzed *via* Western Blot.

Results: PD-L1 was glycosylated in both JHH6 and HuH7 cells, as evidenced by two distinct bands at ~55kDa and ~35kDa. Higher de-glycosylation was observed in JHH6 (>50%) compared to HuH7 (~40%), suggesting a dynamic glycosylation status across different stages or subtypes of HCC. The turnover rates differed, with glycosylated PD-L1 degrading over 72 hours, while un-glycosylated PD-L1 degraded within 24 hours. Treatment with MG-132 markedly increase PD-L1, indicating its susceptibility to proteasomal degradation. Inhibition of AURKA using AK-01 significantly downregulated PD-L1 expression, particularly in the mature forms, whereas si-AURKA took 144 hours to achieve a comparable level of downregulation. PD-L1 silencing reduced PD-L1 protein levels and simultaneously lowered AURKA levels, suggesting a potential feedback loop mechanism.

Conclusion: The differing glycosylation patterns between JHH6 and HuH7 cells may reflect the heterogeneous glycosylation in HCC. Additional HCC models are essential to uncover potentially distinct PD-L1 half-life profiles. Understanding PD-L1 stability and degradation mechanisms in HCC provides valuable insights into PD-L1 functionality, supporting more targeted treatment strategies. Consequently, combination therapy with AURKA and PD-L1 inhibitors presents a promising approach for HCC treatment.

PO2-05-YI
Characterization of SLU7 silencing as a new strategy to treat cancer

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Background and aims: We have previously demonstrated that cancer cells from different origins, including hepatocellular carcinoma (HCC), melanoma, lung, colon or breast cancer, depend on the splicing factor SLU7 for their survival. Mechanistically, SLU7 knockdown-induced apoptosis is preceded by oxidative stress, the accumulation of R-loops and the induction of transcription dependent genomic instability, DNA damage and replicative catastrophe, events accompanied by the accumulation of multiple aberrant splicing isoforms as well as alterations of DNA methylation and transcription. Therefore, we hypothesize that SLU7 silencing could represent a good strategy to treat cancer.

Method: To evaluate the effect of SLU7 silencing on tumor growth, we generated stable mouse HCC Pm299L cells in which SLU7 expression can be knockdown upon doxycycline (DOX) treatment through two inducible systems: iCRISPR/Cas9 gene edition (Pm299L-iCas9-sgSLU7) and shRNAi (Pm299L-shSLU7i). Pm299L-iCas9-sgNTC and Pm299L-shNTC cells were also generated as control. Cells were injected subcutaneously into the flanks of 6-week-old female C57BL/6J and nude BALB/c mice. Once tumors were established, DOX was administered daily by gavage. Additionally, subcutaneous tumors were established with Pm299L, 4T1 mouse breast cancer, and HCT116 human colon cancer cells. Specific mouse or human SLU7 siRNAs in nanoparticles (SilenceMag™ Transfection Reagent), were delivered to the tumors.

Results: In vitro studies verified reduction of SLU7 protein levels after DOX treatment in Pm299L-iCas9-sgSLU7, accompanied by SLU7 gene edition, and Pm299L-shSLU7i cells. As expected, the viability of those cells was compromised, as previously demonstrated upon SLU7 knockdown by siRNA. In vivo, DOX administration to immunocompetent mice with established Pm299L-shSLU7i xenograft tumors led to a significant reduction in tumor volume (p-value 0.0495) compared to PBS administration. Additionally, SLU7 edition via DOX administration in established Pm299L-iCas9-sgSLU7 xenograft tumors in immunocompetent and immunocompromised mice resulted in notable decreases in tumor volume ($p = 0.0002$ and $p = 0.0008$) and tumor weight ($p = 0.0002$ and $p = 0.0002$), compared to PBS. In contrast, DOX administration did not reduce the size of Pm299LiCas9-sgNTC tumor xenografts. Importantly, we also assessed the therapeutic potential of delivering SLU7 siRNAs in magnetic nanoparticles into established xenograft tumors. Intratumoral SLU7 silencing led to a significant growth reduction of not only mouse Pm299L HCC tumors, but also of xenograft tumors induced with mouse breast cancer cells (4T1) and human colon cancer (HCT116), demonstrating the widespread antitumoral effect of SLU7 silencing.

Conclusion: Taken together, our data confirm that SLU7 silencing in vivo could be considered as a potential antitumoral strategy.

PO2-8-YI
Novel chemotherapy selectively induces double-strand DNA breaks and death in naïve and Cisplatin-resistant cholangiocarcinoma tumours

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Background and aims: Cholangiocarcinoma (CCA) comprises a heterogeneous group of biliary malignant tumours characterized by dismal prognosis. Current first-line chemotherapy, including Cisplatin and Gemcitabine, provides limited survival benefits due to the development of chemoresistance. Cisplatin induces single-strand DNA breaks, activating DNA repair mechanisms that diminish its effectiveness. In this study, we present the design, chemical synthesis, and therapeutic evaluation of a new generation of chemotherapeutic agents with unique polyelectrophilic properties, capable of inducing high frequency of double-strand DNA breaks, thereby inhibiting DNA repair and promoting cancer cell death.

Method: We designed, chemically synthesized, and therapeutically evaluated two novel chemotherapeutic agents, Aurkine 16 and Aurkine 18. Their binding and damage to DNA were characterized by Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), comet assay, and electrophoretic mobility of the pUC18 plasmid. Their antitumour effects were tested in human naïve and cisplatin-resistant CCA cells, cancer-associated fibroblasts (CAFs), and healthy cholangiocytes as controls, as well as in xenograft models of CCA.

Results: Aurkines effectively induced double-strand DNA breaks, leading to increased DNA damage and elevated levels of reactive oxygen species, resulting in greater cytotoxicity compared to Cisplatin in CCA cells. Unlike Cisplatin, Aurkines did not activate key proteins involved in single-strand DNA repair, such as ATR and CHK1 phosphorylation. Importantly, these compounds also triggered apoptosis in Cisplatin-resistant CCA cells and CAFs while exhibiting no harmful effects in healthy cholangiocytes, demonstrating malignant selectivity. Additionally, Aurkines demonstrated cytotoxicity in other Cisplatin-resistant cancers, such as breast and ovarian cancer. This selective action against malignant cells was attributed to differences in histone deacetylase (HDAC)-dependent DNA packaging between normal and cancer cells. *In vivo*, Aurkines effectively halt naïve and Cisplatin-resistant CCA tumour growth without adverse effects. Transport studies revealed that Aurkines were selectively taken up by transport proteins OCT1, OCT3, CTR1, and OATP1A2, whereas Cisplatin only modestly utilizes CTR1.

Conclusion: Aurkines represent promising therapeutic candidates for both naïve and Cisplatin-resistant cancers due to their unique polyelectrophilic properties and selective targeting of cancer DNA.

PO2-9-YI
Optimising an ex-vivo pre-clinical model of hepatocellular carcinoma (precision cut tumour slices) to study tumour biology and drug sensitivity

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Background and aims: Hepatocellular carcinoma (HCC) is an aggressive form of liver cancer that unfortunately results in over 5000 deaths annually across the UK. Due to the nature of HCC disease progression many patients only become symptomatic, and therefore diagnosed, during advanced stages of the disease whereby therapeutic options are severely limited to systemic therapies such as monoclonal antibodies, tyrosine kinase and immune-checkpoint inhibitors. Despite substantial research efforts during the past five years patient treatment response rate remains as low as 30% therefore, it is vital we advance our understanding of the HCC tumour microenvironment and its role in therapeutic response. One major challenge during drug development is the lack of preclinical models that fully recapitulate the HCC microenvironment: 2- and 3-dimentional tumour models lack immunological cell types, vital cell-cell interactions, and a complex cellular microenvironment. While the use of *in- vivo* murine experiments can overcome these limitations, they are time-consuming, costly, involve large quantities of animals, and more often than not cause pain and distress to the animals.

Method: Therefore, we have developed a precision cut tumour slice (TPCS) model, generated from orthoptic tumours, that represents a valuable and immunologically relevant tool for studying HCC whereby the tumour microenvironment and immune cell composition can be preserved.

Results: Through the optimisation of culture conditions, including media type, supplements, and platforms, we have developed an *ex-vivo* system that maintains both tumour cell viability and 3-dimentional architecture for up to 10 days of culture. Scaling the model to a 96-well system provides a simplistic and highly flexible approach whereby murine and human cancers, including their cellular and non-cellular compartments, can be studied and utilised as a high-throughput drug screening platform. A library of 26 drugs at two doses were applied to the TPCS model, generated from only 5 orthotopic tumours, which identified two small molecules, salinomycin and rottlerin, that exerted potent anti-tumour activity against HCC.

Conclusion: Furthermore, our model will drastically reduce the number of animals used during drug development by over 90-fold; aligning with the principles of the 3R's (Replacement, Reduction, and Refinement). The model also has the potential as a precision medicine tool, whereby patient-derived tumours can be cultured, and various therapies can be applied and studied to help predict patient responses.

PO2-10-YI
TROP2 as a potential therapeutic target in cholangiocarcinoma

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Background and aims: Cholangiocarcinoma (CCA), the second most common primary liver cancer, is associated with a poor prognosis and has a rising global incidence, urgently requiring effective therapeutic options. Human trophoblast cell-surface antigen 2 (TROP2) is expressed in various cancers and is an emerging target for therapeutic approaches including antibody-drug conjugates (ADCs), such as Sacituzumab Govitecan (SG). This study evaluates TROP2 expression and its role in tumor development and chemoresistance in CCA.

Method: TROP2 expression was detected in human CCA cell lines and patient-derived organoids (PDOs) using flow cytometry. TROP2 expression levels in CCA cell lines were assessed following chemotherapeutic treatment. Knockout of *TROP2* in CCA cell lines was created using CRISPR/Cas9, while overexpression of *TROP2* was established via cDNA-introduced plasmids. Cell lines with *TROP2* manipulation were treated with chemotherapy. PDOs were treated with SG. Cell viability was determined *in vitro*. Immunohistochemical staining (IHC) for TROP2 was performed on a tissue microarray (TMA) comprising 435 CCA patient samples to correlate TROP2 expression with clinicopathological parameters and patient survival outcomes. TROP2 positivity was defined as staining in at least 10% of tumor cells, with an intensity score ranging from 1 to 3 (Bardia et al., 2017). TROP2 expression was classified as low for IHC scores of 0 and 1, and high for IHC scores of 2 and 3. RNA sequencing data from a CCA cohort (n = 122) of the DKFZ/NCT/DKTK-MASTER trial were analyzed and served as a validation cohort.

Results: CCA cell lines and PDOs showed heterogeneous expression of TROP2. Chemotherapy treatment reduced TROP2 levels in CCA cell lines. Increased sensitivity to chemotherapy was observed following *TROP2* knockout, whereas *TROP2* overexpression resulted in chemoresistance. The efficacy of SG correlated with TROP2 expression level. IHC revealed TROP2 positivity in 81.84% of CCA patient samples. In the TMA cohort, high TROP2 expression correlated with poor survival (p = 0.0225, HR 1.37, 95% CI 1.0 - 1.8). This association was further validated in the DKFZ/NCT/DKTK-MASTER cohort, where high TROP2 expression also correlated with poor survival (p = 0.0383, HR 1.7, 95% CI 1.0 - 2.0).

Conclusion: TROP2 is expressed in the majority of CCA cases, providing a strong rationale for TROP2-targeted therapies. In particular, CCA patients with high TROP2 expression may benefit significantly from such treatments due to TROP2's role in chemoresistance and its association with poor survival.

PO2-11-YI
Cabozantinib-Induced Mitochondrial Activation of the cGAS-STING Pathway Enhances the Antitumor Immunity in Experimental Hepatocellular Carcinoma

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Background and aims: Mitochondrial DNA (mtDNA) from damaged mitochondria can act as damage-associated molecular patterns (DAMPs) and induce innate immune responses. dsDNA detection is mainly driven by the cyclic GMP-AMP synthase (cGAS)-STING pathway which by impacting immunomodulation may control tumor progression. Although immunotherapy is the first-line option for hepatocellular carcinoma (HCC), enhancing tyrosine kinase inhibitor (TKI) efficacy remains crucial for many patients. Since cabozantinib induces mitochondrial damage in liver cancer cells, our aim was to analyse STING stimulation as therapeutic strategy to enhance cabozantinib efficacy in experimental HCC.

Methods: Subcellular fractions were obtained by a digitonin-based buffer and confirmed by western blot (WB). mtDNA leakage was assessed by immunofluorescent staining and qPCR in Hep3B cells after cabozantinib treatment. The nucleoside analog 2',3' dideoxycytidine (ddC) was used for mtDNA depletion. mtDNA levels were adjusted for nuclear DNA levels and analyzed using the ΔCT method. cGAS-STING pathway activation and interferon stimulated genes (ISGs) signaling was measured by qPCR and WB. Cell viability with STING modulators was evaluated by MTTs and 3D spheroid models. *In vivo*, the anti-tumor efficacy of cabozantinib and/or vadimezan, a STING agonist, was evaluated using a subcutaneous Hepa1-6 injection mouse model. Tumors were analyzed by immunohistochemistry, WB and qPCR.

Results: Cabozantinib-induced mitochondrial damage in Hep3B cells promoted mtDNA leakage into the cytosol, in a dose- and time-dependent manner, along with increased expression of several ISGs downstream cGAS-STING pathway. Additionally, WB showed increased protein levels of pTBK-1, pIRF3 and nuclear translocation of p65 and IRF3 after cabozantinib treatment, confirming cGAS-STING pathway activation. Depletion of cellular mtDNA content in ddC-treated Hep3B cells, significantly reduced cabozantinib-induced TBK1 phosphorylation and ISG mRNA upregulation. Interestingly, while STING inhibition with H151 partially reduced cabozantinib's cytotoxicity, STING stimulation with vadimezan substantially enhanced the anti-tumor effect, as observed in 2D and 3D Hepa1-6 cellular models. *In vivo*, cabozantinib in combination with vadimezan significantly reduced tumor growth in an immunocompetent mouse model, with increased mtDNA/nDNA ratios detected in serum. Moreover, vadimezan increased granzyme B and $CD8^+$T cells levels in cabozantinib-treated mice, revealing increased presence of activated cytotoxic T cells in treated tumors.

Conclusion: Cabozantinib induces mtDNA-dependent cell death via cGAS-STING signalling in hepatoma cells. STING stimulation enhanced cabozantinib efficacy. Our results, emphasize the role of the cGAS-STING axis in TKI therapies and its agonism as a promising strategy for HCC treatment.

PO2-14
Inhibition of Strawberry Notch 1 represses hepatobiliary carcinoma development

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Background and aims: Notch signaling has been identified as a key factor for the development of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA). Knockout of strawberry notch (sno) mimics loss of Notch in Drosophila, but the role of the human homologue Strawberry Notch 1 (SBNO1) remains unclear. Here, we elucidated the role of SBNO1 in HCC and CCA development.

Method: Expression of SBNO1 was analyzed in large HCC and CCA gene expression and proteomics datasets. Tissue microarrays were subjected to immunohistochemical staining for SBNO1 protein expression. For functional analyses, SBNO1 was inhibited using siRNA or sgRNA followed by cell viability, colony formation and migration assays and gene expression analysis. BioID was employed to identify interaction partners of SBNO1. Syngeneic mouse models using Hep55.1C cells and hydrodynamic tail vein injection (HDTV) were applied to study SBNO1 in vivo.

Results: SBNO1 protein was significantly increased in HCC and CCA compared to non-neoplastic controls. SBNO1 protein to the nucleus localized to the nucleus suggesting its involvement in gene regulation. Inhibition of SBNO1 significantly repressed cell viability, colony formation, and migration and induced distinct expression patterns in HCC and CCA cell lines. However, BioID revealed that SBNO1 similarly modulates gene regulation in HCC and CCA by binding to general transcription factors TAF4 and TAF3. Deletion of Sbno1 reduced tumor growth in syngeneic Hep55.1C tumors and inhibited liver tumor development in vivo applying three different HDTV models of HCC and CCA. In contrast, inhibition of Sbno1 in normal liver did not induce hepatocyte damage.

Conclusion: SBNO1 is a new therapeutic target required for HCC and CCA tumor development in vivo.

PO2-15

Dissecting the role of tumor endothelial cells (ECs) and macrophages in hepatocellular carcinoma (HCC) with VETC+ angiogenesis provides potential targets of treatment

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Background and aims: We investigated the features of macrophages in VETC+ HCC phenotype and the possible interplay between them and ECs, both at morphological and molecular level, in order to provide possible predictive markers and to explore potential targets of treatment

Method: We separated 42 HCCs according to the VETC phenotype (21 VETC+; 21 VETC-) and stained consecutive slides with immunohistochemistry for CD68, CD163 and Tie2. Slides were then scanned and QuPath software used to quantify morphological features, with subsequent statistical analysis (Student's t test, Mantel-Haenszel $\chi 2$ test). We performed spatial transcriptomics in 44 HCC regions of interest (ROIs, 28 intratumoral, 16 peritumoral) derived from 4 VETC+ and 3 VETC- cases; then, we performed DEA, GSEA and GSVA as bioinformatic analysis

Results: Morphological immunohistochemical analysis revealed VETC+ cases are significantly ($p<0.001$) enriched with large, lipid rich CD163+ macrophages, spatially close to ECs; HCC cells significantly overexpress Tie2 with a polarization toward ECs (p:0.002). In CD163+ macrophages, differential gene expression analysis yielded 89 upregulated and 97 genes downregulated in intratumoral ROIs of VETC+ HCC, confirming a distinct profile of macrophages according to the VETC phenotype. Moreover, in VETC+ HCC, they were characterized by a transcriptomic profile similar to the one of pro-tumoral tumor-associated macrophages (TAMs). Finally, in VETC+ intratumoral regions, they showed the upregulation of VEGF signaling pathway

Conclusion: VETC is sustained by a strict morphological relationship between TAMs, ECs and Tie2-expressing HCC; in this scenario, macrophages associated to VETC+ HCC express a specific genetic signature and they exhibit a trascriptomic profile in keeping with TAMs; moreover, they are characterized by the upregulation of specific genetic pathways. Our preliminary data could pave the way to a better comprehension of the complex HCC immune-microenvironment, helping in identifying a biomarker of potential vulnerability.

PO2-16
Pattern of expression, immune contexture and clinical correlates of tertiary lymphoid structures (TLS) in biliary tract cancer (BTC)

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Background and aims: TLSs are proto-lymph node structures found in nonlymphoid tissues, including cancer. As privileged sites for local T cell activation, they are supposed to contribute to mount anti-cancer immunity. Although TLSs have been linked to a favourable prognosis and improved response to immunotherapy in several cancer types, their clinicopathologic relevance in BTC remains elusive.

Method: We retrieved tissue blocks from 82 BTCs undergoing curative-intent surgery between 2002-2022 at the University Hospital of Modena. TLSs were morphologically detected by a trained pathologist on hematoxylin/eosin-stained slides and their spatial distribution (intratumoural vs peritumoural) and abundance were described. Immunohistochemistry for CD4, CD8 and FoxP3 was performed to characterize tumour-infiltrating lymphocytes. The association with clinicopathologic variables was made using the Student T test or Fisher exact test as appropriate and the correlation with survival by Cox regression and Kaplan-Meier analyses.

Results: Among the 82 included patients, the median age was 68 years, 51% (n = 42) had cholangiocarcinoma. Overall, TLSs were detected in 61% (n = 50) of cases, among these TLSs were more commonly located in the peritumoural area (70%, n = 35), followed by both the intratumoural and peritumoural compartment (26%, n = 13) and the intratumoural area alone (4%, n = 2). The number of TLSs ranged from 1-14 with a median of 4. While no significant associations were found between TLSs and patient and disease characteristics, a higher density of TLSs was correlated with increased CD4+ T cell infiltration (p=0.02) and decreased CD8+ T cell infiltration (p = 0.02) in the immune milieu. The presence of TLSs resulted in a significantly prolonged relapse-free (RFS) (median RFS 32,5 vs 17,9 months; p = 0.034) and overall survival (OS) (median OS 43,9 vs 29,3 months; p = 0.032). Likewise, a greater TLSs abundance was linked to a longer RFS (p = 0.02) and OS (p = 0.06). When adjusted for ECOG PS, primary tumour site and disease stage, TLSs remained an independent prognostic factor at the multivariate analysis (p = 0.028).

Conclusion: In this study, we showed that TLSs are present in two-thirds of cases, yet consistent with the immune-excluded nature of BTC, they are more commonly located in peritumoural area. The density of TLSs was associated with a differential infiltration by T cell subsets. The presence and higher abundance of TLSs favourably impact patient outcomes and were independent good prognostic factors in BTCs.

PO2-18-YI
Unveiling SLC2A3 upregulation in intrahepatic Cholangiocarcinoma: insight into metabolic reprogramming and patient prognosis

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Background and aims: Intrahepatic Cholangiocarcinoma (iCCA) is a deadly cancer of the biliary epithelium, characterized by a poor prognosis and limited therapeutic options. Understanding the pathophysiology of iCCA is crucial for developing effective treatments. In the last few years, tumor metabolic reprogramming, a hallmark of cancer, has been shown to play a key role in driving tumor aggressiveness and therapy resistance. This study explores the role of upregulated glycolysis in iCCA and its interactions with tumor aggressiveness and patient prognosis.

Method: Normal cholangiocytes (n=5) and iCCA cells (n=20) were isolated from patients resected at the Humanitas. The cell supernatants were analyzed using mass spectrometry-based targeted metabolomic approaches. RNAseq was performed to identify altered metabolic pathways in iCCA. Immunohistochemistry (IHC) was performed on tissue samples from 40 iCCA patients. Functional assays, including gene silencing, hypoxic culture, migration, and drug treatments, were conducted on iCCA primary cells. Survival analyses were performed on our cohort (n=40) and data from a public dataset (OEP001105; n=167).

Results: The metabolomic analysis of conditioned media revealed that iCCA cells exhibited increased mitochondrial activity and predominantly used glucose and glutamine, while cholangiocytes relied on pyruvate. RNAseq analysis confirmed the significant upregulation of the glycolytic pathway in tumor cells, along with the upregulation of hypoxia and epithelial-to-mesenchymal transition (EMT) pathways. To better elucidate the role of glycolysis, we analyzed the expression of two main glucose transporters, observing a down-regulation of SLC2A1 and a significant upregulation of SLC2A3 in iCCA cells, which was corroborated by IHC analysis. Notably, patients with high GLUT3 expression had significantly shorter disease-free survival and worse overall survival than those with low GLUT3 expression in our cohort, a finding further validated in the external dataset. Moreover, high SLC2A3 expression was associated with increased glucose uptake and activation of several pathways (glycolysis, EMT, hypoxia). Indeed, under hypoxic conditions, low SLC2A3 iCCA primary cells showed increased SLC2A3 expression, glycolytic activity, and resistance to chemotherapy. Whilst the knockdown of SLC2A3 reduced glucose uptake, proliferation and enhanced sensitivity to gemcitabine and cisplatin.

Conclusion: This study reports for the first time that iCCA cells acquire GLUT3 expression during tumorigenesis, which significantly contributes to increased glucose uptake and correlates with poor patient prognosis. Our results underscore the role of GLUT3 in promoting an aggressive iCCA phenotype, highlighting its potential as a therapeutic target for improving treatment outcomes in iCCA.

PO2-19-YI
Blocking RIPK3 impacts mitochondrial dynamics, endoplasmic reticulum stress and inflammation during murine chemical-induced hepatocarcinogenesis

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Background and aims: Receptor-interacting protein kinase 3 (RIPK3)-dependent signalling is triggered under chronic liver injury in humans. We have previously shown that blocking RIPK3 arrested steatotic liver disease progression and ameliorated hepatic metabolic dysfunction in mice. Still, the precise metabolic role of RIPK3 during hepatocarcinogenesis remains elusive.

Method: Two-week-old male C57BL/6 wild-type mice (WT) or *Ripk3*-deficient (*Ripk3*$^{-/-}$) pups were injected with diethylnitrosamine (DEN; 25 mg/kg i.p.). At 42 weeks macroscopic tumours were counted and measured for phenotypic characterization. Gene expression and protein production were evaluated through qRT-PCR and immunoblotting, respectively. Liver samples were processed for immunophenotyping by flow cytometry.

Results: Ablation of *Ripk3* abrogated tumour frequency and tumour size in DEN-exposed mice, compared with WT counterparts. In line with our previous reports, peroxisome proliferator-activated receptor-gamma coactivator-1alpha (*Pgc-1α)*, implicated in mitochondrial biogenesis, was markedly increased in *Ripk3*$^{-/-}$ mice, compared with WT counterparts, in all experimental conditions. In turn, the expression of mitochondrial fission 1 (*Fis1)* and dynamin-related protein 1 (*Drp1*) were decreased in tumour nodules from *Ripk3*$^{-/-}$ mice, consistent with improved mitochondrial quality. Mitochondrial dysfunction is known to be closely related to endoplasmic reticulum (ER) stress, while mitochondria-ER interplay is critical for cell fate and inflammation. In this regard, the absence of *Ripk3* downregulated the three major unfolded protein response pathways in the livers of DEN-exposed mice, as assessed by reduced levels of total activating transcription factor 6 (ATF6), X-box binding protein 1 (XBP1s) and ATF4, as well as both protein kinase R-like ER kinase (PERK) and eukaryotic initiation factor 2 (EIF2α) phosphorylation. Further, *Ripk3* deficiency reduced the expression of the downstream inflammasome markers caspase-1 and interleukin-1β, together with reduced macrophage infiltration in DEN livers. Finally, blocking RIPK3 did not impact the hepatic infiltration of CD4+T or CD8+T cells. Still, the levels of programmed death-1 (*Pd-1*) and its ligand *Pd-l1* were reduced in tumour nodules from mice lacking *Ripk3*.

Conclusion: *Ripk3* deficiency reduced hepatic tumour burden in a murine model of chemical-induced hepatocarcinogenesis. Our results indicate that RIPK3 impacts mitochondrial and ER homeostasis, likely contributing to metabolic dysfunction and inflammation in liver carcinogenesis. Moreover, *Ripk3* deletion influenced the PD-L1/PD-1 axis, dampening T cell exhaustion within the tumour microenvironment.
(Supported by 2021.07666.bd and PTDC/MED-FAR/3492/2021, FCT; LCF/PR/HR21/52410028, “la Caixa” Foundation)

PO2-20-YI
Using patient-led genetics to identify new therapeutic targets in metastatic cholangiocarcinoma

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Background and aims: Cholangiocarcinoma (CCA) includes a diverse range of biliary malignant tumors characterized by dismal prognosis. Tumors are commonly diagnosed when patients present locally-advanced or metastatic disease, limiting the access to potentially curative surgery. The genetic heterogeneity of CCAs has been reported, indicating that the most common gain-of-function mutations in *K-RAS* and *IDH1*, and *FGFR2*-translocations only occur in 16%, 10% and 15% of patients, respectively. These activating mutations are found in a complex genomic landscape where large number of genes are mutated at low frequencies. The role of these heterogeneously mutated genes in CCA progression is unknown and whether these accessory mutations modify the effect of more common mutations to promote tumor metastasis remains to be studied. Here, we investigate the genetics behind CCA dissemination, describing how cell intrinsic changes impact on cancer cell behavior and shape the tumor microenvironment making it more permissive to metastasis.

Method: Analyzing whole genome/exome sequencing data from 277 patients diagnosed with intrahepatic CCA we identified a patient-led list of CCA mutations. We then developed an *in vivo* screen where we expressed gain of function *K-Ras*^{G12D} with a CRISPR/Cas9 gRNA library targeting genes mutated in the patient data. Immunohistochemistry analysis was performed to characterize the primary and metastatic tumors and their microenvironments. Using CRISPR/Cas9-mediated gene silencing, we interrogated whether these low frequency mutations promote epithelial-to-mesenchymal transition, migration and tumor dissemination in human CCA cells.

Results: The in vivo screening showed that *K-Ras* alone was insufficient to initiate tumor formation. In combination with our gRNA library, however *K-Ras*-driven tumors developed within 8 weeks. Exome sequencing of these tumors identified CRISPR-induced mutations in 53 genes which facilitated mutant *K-Ras*-induced tumor growth. We found a range of chromatin modifiers, including *Ncor1*, that upon deletion, together with *Tp53* loss and *K-Ras*^{G12D} overexpression, resulted in metastatic CCA. Although animal survival and tumor burden were not significantly different compared to those harboring *K-Ras*^{G12D};*Tp53*$^{-/-}$ tumors, *K-RasG12D*;*Tp53*$^{-/-}$;*Ncor1*$^{-/-}$ mice presented tumors in various sites other than the liver. Importantly, histological analysis of the primary tumors with metastatic potential revealed phenotypic changes in cancer cells as well as differences in the stroma. Furthermore, deletion of NCOR1 in human CCA cell lines increased their migratory capacity.

Conclusion: Low-frequency mutations in chromatin modifying genes, such as *Ncor1*, cooperate with common driver mutations to promote tumor progression and dissemination by altering cancer cell properties and modifying the tumor microenvironment.

PO2-22
OGT and c-Myc promote non-canonical activation of gene expression by EZH2 in hepatocellular carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) is the most frequent form of liver cancer. The histone methyltransferase (HMT) EZH2 is frequently upregulated in HCC tissues and its expression correlates with HCC aggressivity and poor prognosis. As the catalytic subunit of PRC2, the canonical activity of EZH2 is to trimethylate H3K27 to mediate gene repression. In cancer, EZH2 can have non canonical roles including activation of gene expression in a PRC2- and/or HMT-independent manner. EZH2 is regulated by post-translational modifications, including O-GlcNAcylation by OGT, an enzyme that is also upregulated in HCC. We showed that OGT co-localizes with EZH2 at the promoter of a set of genes involved in cancer-associated pathways in human hepatoma cells.

Method: Here, we used chromatin immunoprecipitation followed by sequencing (ChIP-seq), RNA-sequencing (RNA-seq), computational analysis and functional studies to assess the role of OGT in the epigenetic and transcriptional regulation of cancer-associated genes by EZH2 in HCC.

Results: Our data indicate that OGT is more frequently associated with activation of gene expression by EZH2 than with EZH2-mediated gene repression. In line with our gene ontology and pathway analysis of EZH2/OGT co-activated genes that showed an enrichment of genes related to cell cycle and cancer pathways, including Myc targets, the majority of promoters with EZH2 and OGT co-recruitment also exhibit c-Myc binding.

Conclusion: Collectively, our data uncover that OGT and c-Myc promote non-canonical functions of EZH2 in transformed liver cells and provide important insights for epigenetic strategies as potential future anti-HCC therapies.

PO3-02
Biopsy proteomic profiling predicts long-term HCC risk in patients with Metabolic dysfunction-Associated Steato-Hepatitis

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Background and aims: Metabolic dysfunction-associated steatohepatitis (MASH) is responsible for up to 35% of hepatocellular carcinomas (HCC) worldwide and is the leading cause of liver transplantation for HCC in the United States. Unlike other etiologies, a third of MASH-driven HCC develop in non-cirrhotic livers, thereby escaping screening recommendations restricted to cirrhotic patients, and leading to late diagnosis and poor prognosis. Systematic screening is not feasible due to the high number of patients, and there is currently no predictive marker for HCC development. Tissue proteomic profiling by mass spectrometry is a comprehensive and unbiased approach adapted to these complex issues.
Our goal is to identify a proteomic signature of prediction of MASH-driven HCC and to study pathways involved in carcinogenesis.

Method: We conducted a retrospective study on liver biopsies from 20 MASH patients: 11 patients who developed HCC within 15 years following their initial biopsy (group 1, with a median time from biopsy to HCC of 7.70 years) and 9 control patients who didn't develop any HCC (group 2). The patients were similar in clinical and histological terms.

Results: We obtained a proteomic signature of HCC prediction consisting in 557 significantly deregulated proteins, which allowed differentiation between the two groups. This proteomic signature displayed a very good internal validity assessed by Bootstrap statistical method. It was then validated in a cohort of 13 new patients using two prediction models (random forest and support vector machine) with excellent negative and positive predictive value above 80%. Among these 557 proteins, prothymosin alpha (PTMA) was significantly overexpressed in group 1 patients, with the highest ratio (ratio 16.63). PTMA is overexpressed in many cancers including HCC and is involved in cell proliferation and apoptosis resistance pathways, making it a relevant candidate for carcinogenesis.

Conclusion: Our proteomic signature yields promising results for predicting MASH-driven HCC, highlighting a potential early involvement of PTMA. Its exact role in carcinogenesis is currently tested *in vitro* in a 3D model of MASH developed by the laboratory. Investigating the role of PTMA and other identified targets could open research avenues for preventing tumor development. External validation of this signature in a larger cohort will be necessary before clinical application, in order to optimize HCC screening in MASH patients.

PO3-03
Metabolic liver cancer: Profiling and validation of epigenome-wide DNA methylation markers

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Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most rapidly rising cause of liver cancer (hepatocellular carcinoma, HCC) worldwide, but the underlying molecular processes that drive the development of HCC in the setting of metabolic perturbations are not clear. Aberrant DNA methylation has been associated with the development of different cancers, but their role in metabolic-related HCC (metabolic HCC) is unknown. We aimed to discover and validate epigenome-wide DNA methylation markers associated with metabolic HCC development in a multicenter international study.

Method: We used a case-control design, matching on age (± 5 years), sex, and study site. Epigenome-wide profiling of leukocyte DNA was performed using the Illumina 850k EPIC array. Cell type proportions were estimated from the methylation data, and we obtained data on PNPLA3 genotype from a previous study on some patients. The study samples were split 80% and 20% for discovery and validation. Differential methylation analysis was performed with adjustment for cell type and by using false discovery rate-corrected p-values, volcano plots, and LASSO regression with 10-fold cross-validation, and we generated area under the receiver-operating curves (ROC-AUC).

Results: We enrolled 273 metabolic HCC patients and 316 control patients with metabolic liver conditions without HCC from six sites. Fifty-five CpGs were differentially methylated between cases and controls; 33 upregulated and 22 downregulated in cases. In the validation cohort, the panel of 55 differentially methylated CpGs discriminated metabolic HCC cases from metabolic control patients with AUC = 0.79 (95% CI: 0.71-0.87), sensitivity = 0.77 (95% CI: 0.66-0.89), and specificity = 0.74 (95% CI: 0.64-0.85). Next, we evaluated a multifactorial model that combined the differentially methylated CpGs with diabetes and PNPLA3 genotype and found an AUC = 0.77 (95% CI: 0.67-0.87), sensitivity = 0.80 (95% CI: 0.69-0.92), and specificity = 0.64 (95% CI: 0.50-0.78).

Conclusion: Our results show that a panel of 55 methylation markers differentiates patients with metabolic HCC from control patients with metabolic liver conditions, with a slightly higher sensitivity

when combined with diabetes and PNPLA3 genotype. These methylation markers could be important for risk profiling in the setting of metabolic liver disease and could enhance the development of early detection models for metabolic HCC.

PO3-04
Preventing cholangiocarcinoma progression via a stromal dysregulation and an immune infiltration triggered by cold atmospheric plasma

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Background and aims: Cholangiocarcinoma (CCA) displays a copious desmoplastic reaction and abnormal mechanical properties directed by the cross-talk between cancer cells and tumor microenvironment (TME) cells, at the origin of chemotherapeutic resistance. For overcoming this resistance, innovative therapeutic approaches including locoregional treatments targeting tumor cells and their microenvironment are eagerly awaited. In this line, cold atmospheric plasma (CAP) is a weakly ionized gas that can be designed as a source of cell-damaging reactive oxygen and nitrogen species (RONS). Such plasma source constitutes a promising avenue of research in oncology, providing innovative solutions where conventional techniques seem limited.

Method: CAP was applied to various human cell lines, including CCA tumor cells, cancer-associated fibroblasts (CAFs**)**, and tumor-endothelial cells (TECs), grown in both 2D and 3D (spheroid) models. Cell survival, oxidative stress and pathways were analyzed, along with alterations in cell phenotype and functions by live-imaging microscopy. *In vivo,* ectopic murine CCA was treated with CAP, tumor growth was monitored and TME remodeling, *i.e.* immune and stromal landscape, were analyzed by RNA-seq, flow cytometry and immunostaining. The death of tumor cells in particular immunogenic cell death (ICD) was investigated *in vivo* using vaccination assays and completed *in vitro* by measuring the release of damage-associated molecular patterns (DAMPs) into the extracellular environment of tumor cells.

Results: CAP induces antitumor effects that can be direct (i.e tumor cell death) and indirect (i.e stromal, endothelial and immune cells). *In vitro*, CAP decreases the integrity of a pluricellular spheroid model of CCA and causes stromal dysregulations and immunogenic responses. CAP affects the activation state and migratory phenotype of CAFs and restricts the proliferation and angiogenic profile of TEC**.** *In* vivo, CAP-triggered oxidative stress decreases tumor cell viability and leads to the release of ICD key messengers which stimulate the tumor-surrounding immune cells and promote antitumor immunity. *In vivo*, CAP reduces CCA progression and induces a remodeling of the tumor immune microenvironment by recruiting natural killer (NK) cells to the tumor site.

Conclusion: CAP offers a promising locoregional treatment for CCA by both directly killing tumor cells and indirectly modulating the immune response and disrupting the supportive tumor stroma. It could become an effective tool in enhancing cancer immunotherapy, particularly for cancers with dense fibrotic environments like CCA. To move CAP therapy to clinical application, tests are conducted on large animal using an endoscope to deliver CAP directly into the bile ducts.

PO3-05
Leucine diet targeting leucyl-tRNA synthetase 1 enhances N-glycan biosynthesis translation and overcomes chemoresistance

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Background and aims: Intrahepatic cholangiocarcinoma (ICC) is characterised by high metastatic potential and poor response rates to chemotherapy, with the underlying mechanisms remaining largely elusive. Our study identifies a selective hypotranslation status in ICC and elucidates the regulatory mechanisms, offering insights into potential therapeutic targets to enhance chemotherapy efficacy.

Method: Proteomic data and tumour specimens from ICC patients were utilised to identify key proteins associated with translation and prognosis in ICC. Functional analyses and mechanistic studies were conducted using cell culture, conditional knockout mouse model, and two hydrodynamic transfection ICC models. The effectiveness of chemotherapy, alone or in combination with leucine supplementation or chemical inhibitors, was assessed in vivo.

Results: Leucyl-tRNA Synthetase 1 (LARS1) is markedly reduced in intrahepatic cholangiocarcinoma (ICC), particularly in advanced stages, and its expression is positively associated with patient survival. Using cell lines and diverse ICC models, we demonstrate that LARS1 plays a pivotal role in regulating tumour progression and resistance to chemotherapy. Mechanistically, LARS1 deficiency suppresses the translation of ALG3, ALG12, and RFT1 mRNAs, leading to reduced N-glycosylation of ABCC1 at N929. This alteration enhances ABCC1's function in mediating chemoresistance. Remarkably, exogenous leucine supplementation safely upregulates LARS1 expression and significantly improves the efficacy of chemotherapy in ICC preclinical mouse models.

Conclusion: Our findings uncover new insight into the selective mRNA hypotranslation observed in ICC and mechanisms regulating N-glycosylation at the layer of mRNA translation, providing a diet-drug combined strategy to improve chemotherapy efficacy. These results highlight a promising diet-drug combination strategy to enhance the efficacy of chemotherapy.

PO3-06
Characterization of the peripheral lymphoid and myeloid compartments in hepatocellular carcinoma patients after combined immunotherapy

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Background and aims: Immune monitoring of hepatocellular carcinoma (HCC) patients receiving immunotherapy may help in understanding mechanisms of response and identifying relevant biomarkers.

Method: Patients in the NASIR-HCC trial received selective internal radiation therapy (SIRT) followed 3 weeks later by the anti-PD-1 agent Nivolumab (Nivo). Clinical Benefit (B) was arbitrarily defined as objective response or stable disease lasting for at least 6 months after SIRT. Leukocyte frequencies and their activation status were evaluated by flow cytometry in peripheral blood samples from 39 patients (22 B and 17 non-B patients) obtained at baseline, before (post-SIRT) and 6 weeks after Nivo (post-Nivo).

Results: Analyses of myeloid cells demonstrated that B patients had higher frequencies of CD14+CD16+ monocytes at baseline ($p = 0.04$) and significant increments of conventional dendritic cells post-SIRT ($p = 0.01$). Higher expression levels of activation molecules CD86 ($p < 0.05$) and PD-L1 ($p < 0.001$) were observed in monocytes of B patients at all time points, associated with longer overall ($p = 0.0043$) and progression-free ($p = 0.0001$) survival. A profound decline in most lymphocyte subsets was observed post-SIRT in all patients (except T regulatory cells in non-B patients). However, recovery of lymphocyte levels was preferentially observed post-Nivo in B patients ($p < 0.01$ for T CD4, T CD8 and NKT cells; $p < 0.05$ for B cells). In addition, only B patients had significant post-SIRT increments in the frequency of T cells expressing the antigen-experienced T cell markers CTLA-4 ($p < 0.001$ for CD4 T cells) and CD39 ($p < 0.0001$ for CD4 T cells; $p < 0.01$ for CD8 T cells).

Conclusion: Our results indicate that a stronger innate and adaptive immunity is associated with clinical benefit from SIRT plus Nivo and unveils new targets to design novel combinatorial strategies for HCC patients.

PO3-09-YI
Differential efficacy of irinotecan-based chemotherapy in distinct mouse strain backgrounds in cholangiocarcinoma models

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Background and aims: Cholangiocarcinoma (CCA) is an aggressive subtype of liver cancer with limited therapeutic options. Despite a lack of phase III evidence, irinotecan-based chemotherapy is commonly used as a second-line treatment for advanced biliary tract cancer, emphasizing the clinical importance of understanding its efficacy in preclinical models. This study investigates the effects of irinotecan and its nanoliposomal formulation, nal-IRI, on tumor progression and drug metabolism across distinct mouse strains.

Method: A murine CCA cell line was injected subcutaneously into both immunocompetent C57BL/6J and immunocompromised NSG (NOD.Cg-Prkdc Il2rg/SzJ) mice to induce tumor formation. In addition, human CCA cell lines were injected into NSG mice to facilitate successful engraftment. Once tumors reached 120 mm^3, C57BL/6J mice were treated intravenously with either vehicle, irinotecan, or nal-IRI (50 mg/kg), while NSG mice received only vehicle or nal-IRI, as preliminary data indicated a similar outcome between irinotecan and nal-IRI. Tumor volume was monitored, and irinotecan levels, as well as its active metabolite SN-38, and the inactive form SN-38-glucuronide (SN-38G), were quantified in tumor, liver, and plasma samples via LC-MS. CES enzyme expression, responsible for converting irinotecan to SN-38, was analyzed by qRT-PCR.

Results: In C57BL/6J mice, treatment with either vehicle, nal-IRI, or irinotecan did not result in a significant reduction in tumor growth. In contrast, NSG mice treated with nal-IRI exhibited a markedly enhanced response, with substantial tumor volume reduction. Notably, also in NSG mice bearing human CCA cell lines, nal-IRI treatment resulted in major tumor regression. LC-MS analysis revealed comparable irinotecan levels between strains; however, SN-38 levels were significantly elevated in NSG tumors, with a similar trend observed in liver and plasma samples, despite comparable CES enzyme expression across groups.

Conclusion: The therapeutic response to irinotecan-based therapies in a subcutaneous CCA model varies decisively depending on the strain background, with enhanced efficacy in NSG mice compared to C57BL/6J mice. The heightened sensitivity to nal-IRI observed in NSG mice correlated with increased SN-38 levels in tumors, potentially due to a reduced conversion to SN-38G. These findings highlight the potential influence of strain background on drug metabolism and therapeutic response, emphasizing the need for careful selection of preclinical models to ensure appropriate interpretation and translation of preclinical findings from mouse models to human applications in CCA treatment.

PO3-10-YI

Pivotal role of Fibroblast growth factor 21 in protecting against liver injury and decelerating hepatocarcinogenesis by regulating oxidative stress response in non-steatotic liver disease

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Background and aims: Hepatocellular carcinoma (HCC) frequently develops from chronic liver disease and is associated with a poor prognosis in advanced stages, highlighting an urgent need for a more detailed molecular understanding of HCC pathogenesis. Recently, Fibroblast growth factor 21 (FGF21) - primarily expressed in the liver - has gained attention as a regulator of metabolic processes and a potential therapeutic target to prevent HCC progression, with FGF21 deficiency promoting tumorigenesis in steatotic liver disease. Interestingly, the observed upregulation of FGF21 in our non-steatotic liver injury model prompts further investigation into its role in liver injury, regeneration, and hepatocarcinogenesis.

Method: *Fah* knockout mice develop liver injury and HCC due to the accumulation of toxic metabolites that cause oxidative stress, which can be efficiently prevented by treatment with nitisinone (NTBC). By applying different NTBC regimens, varying degrees of liver injury were induced in *Fah/Fgf21*$^{-/-}$ mice and *Fah*$^{-/-}$ controls. Livers underwent histologic assessment for tumor incidence and liver injury, with liver transaminases serving as biochemical markers of injury and regeneration capacity evaluated through the proliferation marker Ki67. Differential gene expression and proteomic profiling using mass spectrometry elucidated FGF21-regulated pathways.

Results: In moderate chronic liver disease, loss of FGF21 accelerated HCC development, likely due to increased hepatocyte proliferation and altered metabolism, though the extent of liver injury was not affected. In contrast, under acute severe conditions, FGF21 deficiency markedly increased liver injury, likely via an impaired oxidative stress response. Additionally, *Fah/Fgf21*$^{-/-}$ mice exhibited disrupted energy metabolism particularly in the mitochondrial electron transport chain, leading to impaired liver regeneration capacity following acute severe liver injury. Of note, in the long-term severe injury model, *Fah/Fgf21*$^{-/-}$ and *Fah*$^{-/-}$ mice showed similar tumor rates, suggesting that the protective effects of FGF21 are overwhelmed beyond a certain injury threshold.

Conclusion: A comprehensive molecular understanding of HCC development is essential for developing strategies to prevent hepatocarcinogenesis. This study provides evidence for a context-dependent role of FGF21 in non-steatotic liver disease. FGF21 plays a critical role in protecting against liver injury, maintaining regeneration and thereby preventing progression to HCC, depending on the severity of liver injury. Notably, FGF21 analogues like efruxifermin are currently undergoing phase 2b clinical trials for patients with steatotic liver disease, demonstrating therapeutic benefits. Our findings underscore the potential clinical relevance of FGF21 also for high-risk HCC patients with underlying non-steatotic liver disease.

PO3-11-YI
Blockade of CD73 expressed by hepatocellular tumour endothelium improves CD8+ T cell recruitment

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Background and aims: Combining checkpoint inhibitors with vascular targets has shown success in advanced HCC. We conducted RNA sequencing of liver sinusoidal endothelial cells (LSEC) from HCC tumours and matched non-tumour tissue, demonstrating significant differential regulation of immune mediating pathways, including upregulation of CD73. CD73-mediated adenosine production is thought to promote an immunosuppressive tumour microenvironment by limiting immune cell activation and is associated with reduced efficacy of anti-PD1 therapy.[1] LSEC are immune gatekeepers but the function of CD73 on LSEC is less well understood. We therefore sought to explore how LSEC-derived CD73 may affect immune subset recruitment in HCC.

Method: CD73 expression and immune infiltrate in HCC were studied using immunohistochemistry and multiplex imaging. Functional tests of CD73 on immune cell recruitment were performed using static adhesion assays with primary LSEC and peripheral blood lymphocytes, assessing CD4+ and CD8+ recruitment to control or CD73 knockdown (KD) LSEC. Tissue resident memory (TRM) CD8+ were induced, phenotyped using flow cytometry, and assessed in adhesion assays.
The effect of CD73 KD on LSEC was evaluated with bulk RNA sequencing and cytokine array of LSEC secretome. Further analysis of specific targets was performed using PCR and western blot.

Results: Vessels in the HCC tumour capsule uniformly expressed CD73 (n=92), with the tumour capsule often representing the border of the T-cell infiltrate.
CD8+ CD69+ CD103+ TRM phenotype was induced following treatment with IL-15 and TGF beta. CD73 knockdown on human LSEC significantly increased CD8+ lymphocyte recruitment ($p < 0.05$), and reduced CD8+ TRM adhesion ($p < 0.05$).
RNA sequencing of CD73 KD LSEC showed significant upregulation of *HMOX-1*, encoding haem oxygenase-1 (HO-1), a key player in responses to oxidative stress and inflammation. Secretome analysis revealed increased secretion of angiopoietin-2 (Ang2) by CD73 KD LSEC into the culture media.

Conclusion: CD73 KD on LSEC significantly increased CD8+ adhesion although had the opposite effect on CD8+ induced with a TRM phenotype. This specific effect on CD8 effector cells may be mediated by induction of HO-1 expression and Ang2 release in addition to reduced adenosine production. HO-1 upregulation is associated with improved survival in HCC [2] [3], while Ang2 is known to promote leukocyte adhesion via TNF alpha sensitisation.[4] Therapeutic targeting of CD73 could therefore improve CD8+ lymphocyte recruitment in HCC and augment the anti-tumour effect of current systemic therapy.

PO3-12-YI
Epigenetic regulation of liver endothelial cells as a novel target to boost immunotherapy efficacy in hepatocellular cancer

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Background and aims: Hepatocellular carcinoma (HCC) causes significant morbidity and mortality worldwide and its incidence is expected to be doubled by 2040. The HCC tumour microenvironment has been shown to play a significant role in tumour initiation and spread via the regulation of the immune infiltrates in and around the tumour. The endothelium, as part of the tumour microenvironment, is the key to immune cell transmigration and is understood to undergo significant phenotypic and functional changes in response to a number of tumour-associated stimuli. These endothelial changes are regulated by a host of transcriptional, metabolic and epigenetic factors. Here, we aim to study epigenetic regulation of liver endothelial cells as a novel target to boost immunotherapy efficacy in hepatocellular cancer.

Method: Endothelial cells from human HCC and matched non-tumour liver tissues were isolated and RNA sequencing (RNAseq) was performed. Expression of EHMT2 in tumour endothelium in HCC tissues and in isolated primary liver sinusoidal endothelial cells (LSECs) was studied through immunohistochemical and immunocytochemical studies. Next, liver endothelial cells were treated with tumour-associated chemical and mechanical factors, such as inflammatory cytokines, growth factors, shear stress and hypoxia and mRNA expression of EHMT2 was studied. Protein level expression of EHMT2 was studied in response to transforming growth factor beta 2 (TGF-beta2) through western blotting. Graphpad prism 10.5.3 was used for statistical analysis.

Results: Paired analysis of tumour and non-tumour endothelia highlighted that a druggable epigenetic regulator, EHMT2 (euchromatin histone methyl transferase 2) was significantly upregulated in RNA-seq data. Endothelial expression of EHMT2 was confirmed in HCC tissue sections through dual colour-immunofluorescence using HCC-specific endothelial marker (CD34). Liver endothelial cells showed downregulation of EHMT2 expression in response to lipopolysaccharide (LPS) treatment whereas hypoxia treated LSECs showed increased mRNA level expression of EHMT2. A protocol was devised to induce endothelial to mesenchymal transition in isolated LSECs and it showed upregulation of adhesion markers, ICAM1, and chemokine CXCL10 after 72 hours treatment of TGF-beta2.

Conclusion: EHMT2 is upregulated in HCC tumour endothelium and undergoes significant changes in expression in response to different mechanical and chemical biofactors present in tumour microenvironment. As epigenetic changes are dynamic, reversible and druggable, these qualities might provide a novel target to boost immunotherapy efficacy in HCC.

PO3-14-YI
Role of post-translational modification (PTM) in liver cancer: HuR SUMOylation

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Role of post-translational modification (PTM) in liver cancer: HuR SUMOylation

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Background and aims: Posttranslational modifications (PTMs) of RNA-binding proteins like Hu antigen R (HuR) are essential for regulating various cellular processes, including those involved in cancer progression. HuR is a key protein in liver cancer (HCC) and has been implicated in controlling cell survival, proliferation, and invasion. However, the role of SUMOylation (a type of PTM) in regulating HuR function and its influence on liver cancer progression remains poorly understood. This study aims to investigate the impact of HuR SUMOylation on liver cancer cell behavior, particularly in relation to senescence, mitochondrial function, and the potential therapeutic implications of modulating HuR SUMOylation.

Method: In this study a comprehensive SUMO interactome analysis was conducted via GST-tagged SUMO Binding Entities (SUBEs) protein pulldown in several human hepatoma cell lines and in liver tumors from human and mice. Human hepatoma cells were injected into mice to assess tumor growth and senescence marker expression in xenograft tumors.

Results: Here, we show that SUMOylation of HuR promotes major cancer hallmarks, namely proliferation and invasion, whereas the absence of HuR SUMOylation results in a senescent phenotype with dysfunctional mitochondria and endoplasmic reticulum. We employed palbociclib to induce senescence in human hepatoma cells. We found that SUMOylated HuR evades palbociclib-mediated senescence by increasing HuR and global SUMOylation levels in human hepatoma cells. Furthermore, xenograft tumors from human hepatoma cells lacking HuR SUMOylation sites show delayed growth and expression of senescence protein markers in mice.

Conclusion: The SUMOylation of HuR plays a critical role in regulating liver cancer progression by promoting cell proliferation and invasion while protecting cells from undergoing senescence. Overall, SUMOylation constitutes a mechanism of HuR regulation that could be potentially exploited as a therapeutic strategy for liver cancer.

PO3-15
Analysis of the peripheral immune context by single-cell transcriptomics in patients with hepatocellular carcinoma treated with immunotherapy

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Background and aims: Immunotherapy combinations have improved prognosis of hepatocellular carcinoma (HCC) patients eligible for systemic therapy, with approximately 20% showing durable radiological responses. Factors associated with response or resistance to these treatments to optimize patient selection and customize systemic therapies, remain unclear. In this study, we focused on first-line treatment with Atezolizumab (anti-PDL1) and Bevacizumab (anti-VEGF) (atez/bev).

Method: cfDNA (Cell-free DNA) levels were quantified and the C228T mutation in TERT promoter was analyzed in 11 HCC patients treated with atez/bev. Peripheral CD45+ cells were sorted and labeled with a panel of 30 immune antibodies (AbSeq Immune Discovery Panel). Subsequently, cells were isolated individually using the Rhapsody system and 400 genes were amplified. Cell annotation was performed using Celldex and the Monaco reference dataset.

Results: 72.7% of patients were male (8/11) with a median age of 64 years. The most prevalent underlying liver disease was hepatitis C virus infection (54.5%). The median follow-up period was 19 months. During follow-up, 2 patients (18.2%) exhibited a complete radiological response (CR), 5 (45.4%) showed partial radiological response (PR), and 4 (36.4%) developed progression of disease (PD) as a best radiological response, assessed by mRECIST. Patients who exhibited any response (CR/PR) had lower baseline levels of circulating cell-free DNA (cfDNA), with a mean of 3.95±1.96 ng/µl compared to 5.80±1.69 ng/µl in patients with PD. No significant differences were observed in TERT C228T mutation levels between patients who achieved CR/PR (14.06±6.04) or PD (13.60±12.15). Analysis of cell populations in peripheral blood revealed 17 different clusters of cells in our cohort. Patients with PD as best radiological response exhibited a baseline level of 0.56% [0.37-0.83] of plasmablasts, compared with 0.19% [0.13-0.55] in patients with CR+RP (p=0.03). CR/PR patients exhibited alterations in the Th1/Th17 lymphocyte population between the baseline measurement (4.3% [1.19-5.29]) and the three-month follow-up (2.9% [0.79-3.3]) (p=0.03)

Conclusion: In this preliminary analysis, significant differences in certain cell populations implicated in immune response were observed using single-cell technology. This approach could be a useful tool to identify patients with distinct expected outcomes to atez/bev treatment. Deeper molecular studies are currently in progress.

PO3-16
DNA replication stress as a potential therapeutic target in hepatocellular carcinoma

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Background and aims: While hepatocellular carcinoma (HCC) is recognized as the predominant form of primary liver cancer and one of the most prevalent cancers worldwide, there is still a lack of biomarkers that can predict the effectiveness of systemic therapeutic strategies. One potential biomarker, which could be a promising starting point for systemic treatment options, is DNA replication stress. DNA replication stress encompasses the slowing or stalling of replication fork progression and/or synthesis. Indeed, DNA replication stress has been shown as a molecular driver of hepatocarcinogenesis, independent of the etiology. Thus, our research aimed to investigate the potential of DNA replication stress as a biomarker and a potential therapeutic target.

Method: We established a transcriptomic-based DNA replication stress signature, specific for HCC, using an external dataset. In a second step, we developed patient-derived HCC organoids (HCC-Org) by implementing a novel in-house protocol. Then, we tested the sensitivity of HCC-Org to DNA replication stress- and DNA damage repair (DDR)-targeting compounds as well as the standard of systemic treatment for HCC patients. After interrogating the association between the DNA replication stress signature and drug response of HCC-Org, we investigated DNA replication stress and DNA damage in HCC-Org by implementing single-cell and single-molecules assays (e.g. DNA fibers spreading assay, comet assay).

Results: HCC exhibited heterogenous scores among the novel DNA replication stress signature in an independent dataset, with worse survival for patients with higher scores. Moreover, HCC-Org showed differential viability to DNA replication stress- and DDR-targeting compounds such as ataxia telangiectasia and Rad3 related (ATR) inhibitor. At the molecular level, HCC-Org showed a slowdown of the DNA replication fork speed, compared to non-tumoral spheroids, and a concomitant boost of origin of firing, while still showing heterogeneity among organoids originating from different patients.

Conclusion: DNA replication stress- and DDR-targeting compounds might leverage vulnerabilities in HCC-Org, depending on the adaptation of the replication machinery, and offer a potential systemic treatment for HCC patients.

PO3-17
Targeting ATR to overcome cisplatin resistance in hepatoblastoma

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Background and aims: Hepatoblastoma (HB) is the most common pediatric liver cancer. Although the standard treatment, primarily based on cisplatin, achieves an 80% success rate, it remains ineffective for patients who develop chemoresistance, and there is currently no established second-line treatment for these patients. This study aimed to identify new therapeutic options for cisplatin-resistant HB.

Method: Molecular profiling on 14 HB cell lines and one pediatric hepatocellular carcinoma cell line was performed. A subset of eight of these cell lines was then screened with 101 pharmacological compounds as monotherapies and 58 in combination in monolayer cultures. The most synergistic drug combination was subsequently validated in 12 HB spheroid models and two chemoresistant subcutaneous xenograft HB models.

Results: Our cell line panel accurately replicated key driver genomic alterations of HB and predominantly represented the most aggressive cisplatin-resistant molecular subtype at the transcriptomic level. This subtype exhibited strong overexpression of all DNA repair pathways, likely contributing to cisplatin resistance. We identified ATR as a critical target for reversing this resistance. The highly potent ATR inhibitor elimusertib demonstrated the highest level of synergy with cisplatin in both 2D and 3D cultures, as well as, in xenograft models of cisplatin-resistant HB, with manageable toxicity profiles. This combination promoted tumor cell death by increasing DNA damage and activating p53. Elimusertib also showed synergy with other HB chemotherapeutic agents, including carboplatin, irinotecan and/or vincristine and demonstrated ability to inhibit mTOR.

Conclusion: This study highlights the crucial role of ATR in mediating cisplatin resistance and demonstrates the potent cytotoxic effects of the elimusertib/cisplatin combination in cisplatin-resistant HB models. These findings identified a promising new therapeutic option for treating chemoresistant HB.

PO3-18
Sex shapes microbiota-epigenetics interplay in HCC development rewiring mitochondrial dynamics

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Background: Hepatocellular carcinoma (HCC), the most frequent primary malignancy of the liver, is among the leading causes of cancer-related death worldwide. The vast majority of HCCs is associated with chronic liver disease due to a known underlying aetiology, including chronic viral hepatitis (B and C), alcohol intake and non-alcoholic steatohepatitis (NASH). It has been reported that epigenetics plays an important role in liver oncogenesis and gut microbiome changes in HCC patients, but the relationship between them over the course of carcinogenesis is not clear. Finally, epidemiological reports indicate that the incidence of HBV-related HCC is higher in males and postmenopausal females than other females, but the cause underlie this notion is largely unknown.

Aims: The interplay between the transcriptomic profiles and the microbiome in the hepatocarcinogenesis of MYC/X mice model.

Method: WT and X/Myc double transgenic mice, expressing in the liver both HBV HBx under control of viral regulatory elements and c-myc, were characterized by liver tumor (T) and peri-tumor (PT) RNA-seq (75x2 Novaseq Illumina). 16s rRNA of mice stools was sequenced by Miseq Illumina platform at 10 and over 20 months, during liver carcinogenesis and at the development of HCC respectively.

Results: Integrative transcriptome analysis of T and PT indicated that X/MYC mice correlated with human subclass S2 HCC, characterized by proliferation as well as stemness. RNA species of WT, PT and T tissues showed a different transcriptomic profile depending on the gender. In particular we found a different pattern of mitochondrial metabolism according to sex. We also found by rRNAs 16s results that in the early phases of liver carcinogenesis, an increasing in Gram – bacteria in the male gut are accompanied by growing levels of LPS in the liver of male mice. This major incoming of endotoxin in male tissues matches with a boost of EZH2 methyltransferase in the liver, that in turn regulates the promoter activity of mitochondrial genes in male mice. EZH2 has already been reported to be upregulated in liver cancer, but here we show that this upregulation happens early in the inflammation and that it is different according to sex. To conclude, we defined a specific network of relevant RNA and bacterial species that characterized females and male hepatocarcinogenesis.

Conclusion: Our findings expand the current understanding of how gut-liver axis contributes to liver carcinogenesis by controlling histones tri-methylation and mitochondrial activity and how all this pathway is sex-dependent.

PO4-02
Personalized biomarkers scores outperform established scores in the short- and long-term prediction of hepatocellular carcinoma and decompensation

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Background and aims: Predicting the two main complications of compensated advanced chronic liver disease (cACLD) – hepatocellular carcinoma (HCC) and decompensation - is crucial for improving prognosis. We tested various combinations of serum biomarkers to predict HCC and decompensation at 5- and 10-year in a cohort of cACLD patients, of any etiology and compared the results to the established GALAD, aMAP, and EPOD scores.

Method: From 2011 to 2018, we prospectively recruited 545 patients with cACLD. At enrollment, we analyzed 28 serum biomarkers related to inflammation, immunity, metabolism, coagulation, and angiogenesis (Ella ELISA,Bio-Techne). Based on the most effective combinations in the Cox models, we developed personalized biomarker scores (BioMscores) tailored for each condition and specific subgroups categorized by sex and etiology (viral, MAFLD/MASH, alcohol). Additionally, we calculated GALAD, aMAP, and EPOD scores and assessed their predictive capability for HCC and decompensation, versus the BioMscores using Cox proportional hazard regression models and the AUROC method.

Results: Patients were followed for a median of 3.60 and 5.58 years at 5 and 10 years, respectively. We observed 92 incident HCC cases (16.9%). For the entire HCC cohort, a combination of 9 biomarkers linked to inflammation, IGF-1 transport and uptake, and cellular response to hypoxia outperformed GALAD and aMAP (BioMscore HR 4.700,p<.001; GALAD: HR 1.307, p<.001; aMAP HR 0.997,p=NS). Notably, BioMscores differed significantly between subgroups, with males and MAFLD/MASH patients showing more inflammation, and females showing more hypoxia-linked biomarkers. GALAD and aMAP did not havepredictive ability in MAFLD/MASH, while BioMscore did (HR1.929, p<.001). For decompensation, BioMscores revealed inflammation- and angiogenesis-linked biomarkers in all subgroups except females, who lacked inflammatory biomarkers. BioMscore for the entire group had the same predictive ability as EPOD but greatly outperformed it in males, females, and etiology subgroups. EPOD failed to predict decompensation in MAFLD/MASH, while BioMscore did (HR 4.455, p=.002).

Conclusion: HCC and decompensation displayed significantly different risk profiles: HCC was more closely linked to inflammation, while decompensation was associated with coagulation and angiogenesis. Males predominantly exhibited inflammation-associated biomarkers, whereas females were more likely to have markers related to hypoxia and angiogenesis. Notably, personalized scores significantly outperformed traditional ones in predicting both conditions, achieving a higher degree of accuracy across all subgroups.
(supported by AIRC IG 2020-ID. 24858 project-P.I. Villa Erica; AIFA Independent Research grant TRS-2018-00001238 to Erica Villa).

PO4-05-YI

Circulating tumour DNA analysis for predicting clinical outcomes in advanced liver cancer: insights from tumor fraction, and DELFI score

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Background and aims: Advanced hepatocellular carcinoma (HCC) is associated with poor clinical outcomes, and novel prognostic markers are needed to stratify treatment. Shallow whole-genome sequencing (sWGS) of circulating cell-free DNA (ccfDNA) has shown promise as an efficient and cost-effective method for assessing circulating tumour DNA (ctDNA) fractions, detecting large-scale genomic alterations, and analyzing fragmentomics.

Method: Pretreatment plasma samples from 134 patients with advanced HCC who underwent first-line systemic therapy either tyrosine kinase inhibitors (TKIs) or combination immunotherapy (atezolizumab and bevacizumab, A/B) were included. Tumour fraction (TF) was calculated using ichorCNA software. Fragmentomics analysis evaluated the ratio of short to long DNA fragment length using the DELFI approach. The predictive value of both TF and DELFI scores in relation to progression-free survival (PFS) and overall survival (OS) was evaluated.

Results: 83 patients received TKIs and 51 received A/B. Elevated TF was positively associated with high AFP levels (≥400 ng/ml), BCLC- C, and larger tumour size. Patients with high TF had a significantly shorter median PFS of 3.0 months (95% CI: 2.77–4.7) compared to 10.6 months (95% CI: 2.77–14.61, p<0.001). High TF was also associated with a shorter median OS of 7.8 months (95% CI: 6.13–16.7) compared to 20.2 months (95% CI: 17.27–24.47) for low TF (p=0.021). High DELFI score were associated with poorer outcomes, with a median PFS of 4.9 months (95% CI: 3.3–7.3) compared to 11.1 months (95% CI: 9.57–16.53) compared to low DELFI score (p<0.001). High DELFI score were associated with a shorter median OS of 11.3 months (95% CI: 7.03–14.7) compared to 23.3 months (95% CI: 18.53–29.6) for low DELFI score (p<0.001). In terms of treatment, the TF and DELFI did not show any effect. In Cox regression multivariate analysis, DELFI (hazard ratio (HR) 2.95, 95% CI: 1.22–7.11; p=0.016), BCLC (HR 3.23, 95% CI: 1.21–8.63; p = 0.019) for PFS and DELFI (HR of 4.37, 95% CI: 1.52–12.5; p = 0.006), BCLC (HR of 4.54, 95% CI: 1.35–15.2; p = 0.014), for OS were identified as independent predictors for A/B group.

Conclusion: High TF and elevated DELFI are linked to poorer outcomes in advanced HCC, highlighting their potential as prognostic biomarkers for guiding treatment decisions in future studies

PO4-06-YI
Development of liver cancer-derived organoids for drug screening and disease modelling

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Background and aims: Primary liver cancers (PLC), including hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA), are a heterogeneous epithelial tumor, characterized by drug resistance and poor outcomes. Hence, there is an urgent need for the discovery of promising biomarkers and reliable therapeutic strategies. In this regard, 3D in vitro models have been developed to better understand the molecular mechanisms underlying PLC progression and to provide high-throughput experimental techniques, to assess treatment efficacy. The aim of this study was to establish patient-derived organoids and develop a platform of different molecules that are able to experimentally interfere with tumor cell viability, thus identifying new possible substances for PLC therapy.

Method: The development of patient-derived organoids was obtained mincing and digesting tumor and non-tumor liver biopsies into small cell clusters, which were then seeded into Matrigel domes. Exploiting immunofluorescence and qPCR techniques, PLC-derived and healthy organoids were characterized and then treated with each selected substance at different doses, measuring cell viability thereafter. Furthermore, potential underlying PLC processes that the tested molecules might target were explored.

Results: We successfully developed and characterized PLC-derived and healthy organoids, highlighting major morphological characteristics and verifying the presence of specific markers. Afterward, as a preliminary screening, we assessed the sensitivity of each patient-derived organoid to four anti-cancer substances, including clinically-used or under development drugs. In particular, we started by testing three small molecules of Voltage-Dependent Anion-selective Channel isoform 1 (VDAC1)-antagonist and observed a significant dose-dependent decrease in viability in tumor cells. In addition, we studied the effect of a naturally derived metabolite, Usnic Acid, which induced a reduction of cell viability at high concentrations. Moreover, patient-derived organoids were treated with L-Asparaginase, which did not have any remarkable effect on viability. To better mimic the tumor microenvironment contribution, we also co-cultured liver organoids with PBMCs, in order to evaluate antibody-dependent cellular cytotoxicity.

Conclusion: We developed and characterized a well-defined PLC in-vitro model that allowed us to investigate the effects of multiple substances as potential therapeutic approaches.

PO4-08
Diagnosis of extrahepatic cholangiocarcinoma in the presence of bile duct stricture using proteomic profiling: a proof-of-concept

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Background and aims: The diagnosis of extra-hepatic cholangiocarcinoma (eCCA) remained challenging due to the classical pathological analysis of cytological samples (CS) from the bile duct, which has a sensitivity of only 50%. Additionally, despite advances in omics technologies like Next-Generation Sequencing, these methods still report around 25% false negatives. This study aimed to enhance the diagnostic accuracy of CS using a novel proteomic profiling approach.

Method: We selected a development cohort of 10 patients with CS obtained from biopsies or brushings, including five diagnosed with malignant eCCA and five with benign conditions. Formalin-fixed tissues were analyzed by mass spectrometry to generate a proteomic signature distinguishing these groups. A validation cohort of five patients with indeterminate CS, whose final pathology was confirmed after surgical resection, was utilized to validate the accuracy of the signature.

Results: A total of 2,676 proteins were identified, with 49 proteins significantly deregulated between malignant and benign samples. Proteomic profiles distinguished the two groups, through hierarchical clustering and principal component analysis. Extracellular matrix proteins were mainly involved in the signature associated with key biological functions like cell adhesion and epithelial-mesenchymal transition. Machine learning algorithms and statistical tests achieved perfect matching for the validation cohort.

Conclusion: This study demonstrated the potential of a proteomic profiling approach as a promising diagnostic tool for eCCA. By accurately differentiating between benign and malignant lesions, this signature could significantly enhance clinical decision-making, reduce unnecessary surgical interventions, and improve patient outcomes. Future validation in larger cohorts is essential to confirm its effectiveness.

PO4-09
TLR3 and interferon gamma signaling synergistically increases drug efficacy in cholangiocarcinoma

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Background and aims: Cholangiocarcinoma (CCA) is a common liver malignancy arising from the bile duct epithelium. CCA is usually diagnosed at an advanced stage due to the lack of specific symptoms. Surgery is the only curative option, but patients also receive immuno- chemotherapy, with the combination of gemcitabine, cisplatin and durvalumab being the most effective treatment. Nonetheless, these drugs are often ineffective and fail to improve patient survival. Consequently, novel therapeutic targets or combination therapies are necessary to improve clinical outcomes.

Method: We analyzed gene expression profiles, cytokine, and phospho-protein arrays together with functional readouts upon IFNγ and TLR3 treatments using CCA cell lines combined with tumor 3D cultures and patient-derived organoids. Additionally, we retrospectively analyzed patient genomic data to investigate the relevance of these pathways in patient cohort.

Results: We identified that IFNγ from the tumor environment enhanced immune escape in CCA cells, including the upregulation of TLR3. Engagement of TLR3 using specific ligands together with IFNγ enhanced necroptosis pathway in CCA cells, which could be utilized to enhance sensitivity to gemcitabine + cisplatin treatment. Accordingly, patient-derived organoids that showed resistance to gemcitabine-cisplatin could be successfully eliminated using a combination therapy with IFNγ and TLR3 ligand. At the molecular level, Erk2 activation was key to these effects. Importantly, TLR3, IFNGR1 and downstream pathways were upregulated in CCA tumor samples and correlated with clinical outcomes.

Conclusion: Our data demonstrate that in CCA tumors where T cell activation occurs in the microenvironment, sufficient local IFNγ release can support chemotherapy efficacy. In such cases, treatment could be enhanced by adding TLR3 ligands for improved anti-tumor eradication.

PO4-13
Review of 20 years of liver tumor collection in the French liver biobank network

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Background and aims: Liver cancers (hepatocellular carcinomas, cholangiocarcinomas) are common cancers worldwide, being the third most common cause of cancer mortality. The identification of effective tools for the early detection of liver cancers and of innovative targeted drugs therefore remains a major challenge. The aim of the project was to collect tumor samples and medical information from liver cancer surgery patients in order to provide samples for genomic analyses as well as related data for real-life studies of these patients after surgery.

Method: In the early 2000s, 9 liver centres in France set up a network to organize the exchanges of information and biological materials, requiring standardized procedures for the collection and preservation of liver samples and linked-information related to demographic, clinical, biological and histological data, in line with the OECD recommendations for Biological Resources Centres. Samples are collected and stored locally, whereas related annotations are remotely collected within a central data warehouse. Biological samples and associated data are made available to academic and industrial research groups following acceptance of research projects submitted to the Scientific Committee and the Executive Board of the network.

Results: Since 2002, the network has collected biological samples: paired tumor and non-tumor tissues and blood; and clinico-biological data: 644 clinico-biological variables, among them 307 being mandatory, from 4736 patients who have undergone surgical removal of tumors, including either total (transplanted) and partial hepatectomy, or ablation therapy. The cohort included 86.5% hepatocellular carcinomas, 8.0% cholangiocarcinomas, and 5.5% benign tumors. The aetiologies of the disease included HBV: 9.9/1.7 %, HCV: 16.8/0.1 %, alcohol consumption: 21.3/5.1 %, hemochromatosis: 3.2/0 %, MASH: 10.7/16.8 %, multi-factors: 20.3/10.4 %, respectively in patients with hepatocellular carcinomas and cholangiocarcinomas. The non-tumoral areas were cirrhotic (F4) 46.5 %; fibrotic (F3-F2) 25.1 %; or histologically normal tissue (F0-F1) 28.2 %.

Conclusion: The French liver cancer biobank network has contributed more than 100 papers aimed at identifying key genomic alterations occurring in tumors development, as well as new set of biomarkers, including transcriptomic and proteomic signatures, and candidate targets for anti-cancer drugs. Studies on real-life patient show the value of studying patient characteristics and outcomes over a long post-operative period to identify risk factors and prognostic markers of disease progression. Current challenges include the integration of new data, such as AI-based imaging, lifestyles, environmental exposure etc. Extending the network to European partners should allow us to enrich the data warehouse, while avoiding biases in patient recruitment.

PO4-15-YI

Evaluating microbeam radiotherapy's effect on normal liver tissue: a step towards understanding its potential as a treatment

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Background and aims: Radiation-induced liver disease limits the use of radiotherapy for liver cancer, particularly hepatocellular carcinoma (HCC), which is highly resistant to treatment and prone to metastasis. Microbeam radiotherapy (MRT), a form of spatially fractionated radiotherapy, has shown promise improving normal tissue tolerance (Trappetti, 2022) and has achieved complete tumour responses in glioma and melanoma (Sotiropoulos, 2021; Prezado, 2020; Fernandez-Palomo 2021). This study aims to assess MRT's effects on healthy liver tissue to explore its potential for liver cancer treatment.

Method: C57BL/6 mice were partially irradiated using MRT at the European Synchrotron Radiation Facility and the Australian Synchrotron We delivered microbeams of 400 Gy, with a beam width of 50 µm and spacing of 400 µm from centre-to-centre, and the dose rate approximately 14000 Gy/s at the ESRF and 1000 Gy/s at the AS. Immunohistochemical analyses assessed DNA damage (using γH2AX), cell death (TUNEL assay), proliferation, and fibrosis to evaluate regenerative responses and tissue tolerance.

Results: Within 72 hours post-MRT, γH2AX staining revealed targeted DNA damage along the microbeam paths. Proliferation markers appeared primarily within these paths at 48 hours, as well as in adjacent valley regions, suggesting a regenerative response. Vimentin expression and macrophage infiltration suggested activation of hepatic stellate cells and an inflammatory response. Serum level of liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels showed only mild elevations from 1 to 6 days post-MRT, indicating minimal early injury. At six months post-MRT, liver function was fully restored, with no evidence of fibrosis, highlighting the tissue-sparing potential of MRT.

Conclusion: Our findings suggest that MRT activates early repair mechanisms in liver tissue, preventing long-term fibrosis and preserving liver function post-irradiation. These tissue-sparing effects support MRT's potential as a therapeutic option for liver malignancies. Ongoing studies will evaluate MRT's anti-tumour efficacy and underlying mechanisms to optimize its applications in liver cancer treatment.

PO4-16
Recapitulating the liver tumour endothelium in vitro: a tool for novel drug delivery studies

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Background and aims: Hepatocellular carcinoma (HCC) represents a significant and increasing healthcare burden. Treatment failure is commonly attributed to the highly immunosuppressive tumour microenvironment and key contributors to this are endothelial cells which line the tumour vasculature. Characterising these cells in detail could support the design of much needed novel therapeutics; however, isolating and culturing these cells *in vitro* is extremely challenging. Nevertheless, RNA-sequencing data from our group has demonstrated that HCC tumour endothelial cells possess a distinct phenotype, when compared to endothelia isolated from matched non-tumour tissues. In this project, we investigate the reprogramming of primary human liver endothelial cells to a tumour-like endothelial phenotype to aid the design of novel, targeted drug delivery systems for the treatment of HCC.

Method: Key markers enriched in HCC endothelial cells including CD31, PLVAP, CD73 and stabilin-1 were validated in HCC tumour tissues *ex vivo* via immunohistochemistry. Primary human liver endothelial cells were isolated from human liver tissues, through mechanical and enzymatic digestion, density-gradient centrifugation and immunomagnetic positive selection for $CD31^+$ endothelial cells. Isolated liver endothelial cells were then cultured and treated with a combination of cytokines, angiogenic factors and tumour cell conditioned media. PCR assessed mRNA expression of several tumour endothelial markers, including *CD31*, *CD34*, *NT5E* and *PLVAP,* and immunofluorescent staining and western blotting confirmed the protein expression. RNA sequencing was performed on treated endothelial cells to compare transcripts to tumour endothelial cells data. The recruitment of immune cells across the induced tumour-like endothelium under physiological flow conditions was then investigated by shear-flow adhesion assay with isolated peripheral blood monocytes.

Results: We found that a combination of proinflammatory cytokines IFN-γ, TNF-α and angiogenic factor vascular endothelial growth factor (VEGF) for 24 hours had reproducible effects on liver endothelium, upregulating several factors identified in our tumour endothelial cell RNA-sequencing data. Additionally, the induced tumour-like endothelial cells support the recruitment of peripheral blood monocytes under physiological flow conditions.

Conclusion: Using a treatment with IFN-γ,TNF-α and VEGF, we have managed to induce a liver tumour endothelial-like phenotype in primary human liver endothelial cells attested via qPCR analysis, immunofluorescent staining, western blot, and RNA sequencing analyses. Future studies will investigate the migration of cargo-loaded monocytes across the induced tumour-like endothelium under physiological flow conditions *in vitro*, with the intention of producing a novel drug delivery system to HCC tumours.

PO4-17
Pattern of progression and post-progression survival in intermediate stage hepatocellular carcinoma. An analysis of the TACE 2 trial

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Background and aims: The pattern of progression (POP) following systemic therapy or transarterial radioembolization is a known prognostic variable for post-progression overall survival (PPOS) and hence POP has been proposed as a stratification factor for clinical trials. Its prognostic significance has not been explored within the transarterial chemoembolization (TACE) trial treated population. Here, we analyse the impact of POP on PPOS in patients treated within TACE-2 Trial.

Method: TACE-2 was a prospective, multicentre, randomised, placebo-controlled phase III trial comparing TACE plus sorafenib with TACE plus placebo, with progression-free survival as the primary end-point. Patients with radiological progression were included in this study. POP classification included: target or non-target lesion progression (TNTLP), new intrahepatic lesions (NIH), and new extrahepatic lesions (NEH). PPOS was assessed using Kaplan-Meier method, using log-rank test for comparison. Cox proportional hazards model was used to assess hazard ratios (HR) for death with 95% confidence intervals (CIs). Subgroup analysis compared PPOS in patients receiving TACE plus placebo versus TACE plus sorafenib across these progression patterns. Multivariate analysis was performed, adjusting for these covariates: time from randomisation to progression, albumin, bilirubin, AFP, Modified RECIST, ECOG status, hepatitis C status, and disease focality.

Results: 151 patients were included (43 with TNTLP, 80 with NIH lesions, and 28 with NEH lesions). Median PPOS was 7.0, 13.3 and 7.0 months for TNTLP, NIH and NEH groups respectively. Comparing PPOS relative to TNTLP group, those with NEH lesions tended to have a worse survival (HR 1.47 [95% CI 0.82 - 2.62, p = 0.20]) whilst those in the NIH group had a similar outcome (HR 0.93 [95% CI 0.56 - 1.54 p = 0.78]. Univariate analysis by POP group comparing treatment arm to placebo revealed a difference between the treatment arm of NIH group compared to placebo (HR 0.53 [95% CI 0.28 - 1.00, p = 0.05]). However, this difference disappeared in a multivariate analysis adjusting for other covariates (HR 1.25 [95% CI 0.44 – 3.49, p = 0.68]), showing no significant difference between TACE with placebo and TACE with sorafenib in all POP groups.

Conclusion: This post-hoc analysis demonstrates a trend towards worse survival in patients who progress with new extrahepatic lesions after TACE treatment, but the prognostic implications of progression pattern are not influenced by the addition of systemic therapy in patients treated with TACE. This study was limited by small numbers of patients in each POP group, warranting further prospective evaluation.

PO4-19
Integrated Single-Cell RNA and Chromatin Accessibility profiling reveals epigenetic drivers of tumor heterogeneity in cholangiocarcinoma

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Background and aims: Cholangiocarcinoma (CCA), a malignancy of the bile duct epithelium, poses significant challenges due to late diagnosis, poor prognosis, and limited therapeutic options. While genomic alterations in CCA have been well studied, the role of chromatin accessibility and epigenetic regulators in promoting tumor progression remains underexplored. This study leverages single-cell RNA sequencing (scRNA-seq) and single-cell ATAC sequencing (scATAC-seq) to identify specific epigenetic drivers of tumorigenesis in CCA, focusing on chromatin remodelers, transcription factors, and chromatin accessibility changes.

Method: Here, we performed single-cell multi-omic profiling on 93,187 cells from 8 tumors and 8 matched adjacent non-involved tissues from intrahepatic cholangiocarcinoma (iCCA) patients to identify molecular defects specific to large and small duct-derived CCA.

Results: By integrating single-cell chromatin accessibility with single-cell RNA-seq data, we mapped enhancer-promoter interactions, identifying distinct chromatin regions enriched for the transcription factor motifs of KLF5 and HNF1B in large and small duct-derived malignant CCAs, respectively. Enhancer reprogramming by these transcription factors is specifically associated with genes involved in tumor cell survival, resistance, and metastasis. Additionally, our pseudotime analysis of chromatin accessibility changes revealed a chromatin trajectory of tumor progression, showing progressive chromatin opening in regions linked to tumor status, regulated by key transcription factors and associated with deregulated genes.

Conclusion: Overall, our study provides a framework for understanding the epigenetic mechanisms, including enhancer reprogramming and transcription factor rewiring, that drive tumor progression in CCA.

PO4-20
High-resolution quantitative reconstruction of microvascular architectures in mouse hepatocellular carcinoma models

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Background and aims: Alterations in liver vascularization play a remarkable role in liver disease development, including hepatocellular carcinoma (HCC), but remain understudied. This study evaluated the hepatic microvascular imaging method and provided high-resolution quantitative anatomical data on the characteristics and architecture of liver vasculature in wild-type (WT) mice and HCC mouse models.

Method: C57BL/6 mice were injected with Akt/Ras or Sleeping Beauty transposon to induce HCC. Liver tissues from normal and Akt/Ras mice underwent hematoxylin and eosin, Masson's trichrome, Ki67, and lymphatic endothelial receptor-1 staining. Using cutting-edge high-definition fluorescence micro-optical sectioning tomography, high-precision microvascular visualization of the liver was performed in WT and Akt/Ras HCC mice.

Results: The sectioned volumes of normal and HCC liver tissues were 204.8 mm3 and 212.8 mm3, respectively. The microvascular systems associated with the tissues of Akt/Ras HCC mice were twisted, disordered, and compressed by tumor nodules. In the four tumor nodules, the path of the hepatic artery was more around the tumor edge, whereas the portal vein occupied the central position and constituted the main blood vessel entering the tumors. The porosity of HCC and paracancerous cirrhotic tissues was significantly less than that of normal tissues. The radii of the central vessels in the hepatic sinusoid of paratumoral cirrhotic tissues were significantly higher than those of normal tissues (36.8 vs. 66.4 μm, $P<0.001$); however, the hepatic sinusoid density of paratumoral cirrhotic tissues was lower (154.3 vs. 496 μm^{-2}, $P<0.001$).

Conclusion: This research provides a deeper understanding of the normal liver microvasculature and alterations in cases of cirrhosis and HCC, which complements scientific insights into liver morphology and physiology. This straightforward research approach involving the novel 3D liver microvasculature can be used in multiscale physiological and pathophysiological studies regarding liver diseases.

PO4-22
Development of small molecule degrader of G1 to S phase Transition 1 (GSPT-1) for the hepatocellular carcinoma treatment

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Background and aims: Targeted protein degradation (TPD) has recently revolutionized drug development by enabling the targeting of previously “undruggable" molecular targets providing new treatment options for diseases with unmet medical needs. Molecular glues are a class of compounds that enable TPD, they bind selectively to the ubiquitin ligase, altering its surface and facilitating novel interactions that result in ubiquitination and degradation of previously inaccessible proteins. This approach has recently emerged as a novel therapeutic modality in drug discovery. In this report, we present an in vitro and in vivo characterization of CT-01, a newly developed molecular glue capable of inducing selective degradation of GSPT-1 protein whose degradation leads to an Integrated Stress Response (ISR) and induction of apoptosis in hepatocellular carcinoma cells.

Method: A series of biophysical methods (FP, SPR, AlphaLisa) have been utilized to characterize drug interactions with target protein and E3 Ligase. The biological properties of the reported molecular glue have been determined using cancer cell culture models (cell viability assessment using Cell Titer-Glo Assay), molecular biology techniques (western blots to confirm target protein degradation and the MoA) as well as a hepatocellular carcinoma (HCC) xenograft in vivo models.

Results: CT-01, a molecular glue binds both E3 Ligase and target protein with high affinity and forms a ternary complex in vitro. In cancer cells, it induces the degradation of GSPT-1 resulting in cancer ell death. The compound shows a desirable PK and PD profile, as well as causes in vivo tumor growth inhibition in human HCC xenograft mouse model.

Conclusion: Presented results indicate that targeting GSPT-1 protein by induction of its degradation could represent a new and effective strategy for cancer treatment. CT-01, a molecular glue has just completed preclinical development, and its safety, pharmacokinetics and pharmacodynamics will be evaluated in a phase 1 study commencing in 2025 – an open-labeled, dose escalation and dose expansion study of CT-01 as monotherapy and combination therapy in subjects with intermediate or advanced hepatocellular carcinoma.

PO5-04
Leveraging normothermic liver machine perfusion as a platform to establish tumour directed ex-situ treatment

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Background and aims: Current tumour models fail to accurately reflect the tumour microenvironment, contributing to the high attrition rates of new drug candidates. Therefore, novel tumour models which allow a closer approximation of reality are urgently needed. Normothermic liver machine perfusion (NLMP), a technology originally developed for clinical use in the field of transplant surgery, may be leveraged to serve as a platform for more realistic translational research models. In this proof-of-concept study, we aim to test and refine an adeno-associated virus (AAV) library within the context of a normothermic ex-situ tumour perfusion model. AAVs hold the potential for effective and specific gene-based interventions by serving as vehicles for tumour directed gene delivery.

Method: Hepatectomy specimes were obtained from patients undergoing liver transplantation for HCC. After retrieval, specimens were put on ice, flushed with HTK solution and reconstructed in anticipation of NLMP. Following reconstruction, NLMP was initiated. Biopsies of the HCC and the cirrhotic liver tissue were taken. Viability was assessed with real-time confocal microscopy (RTCM) and standard histology. Precision cut liver slices (PCLS) from cirrhotic and tumour tissue were prepared, cultured and infected with a library of 49 AAV capsids. To assess individual capsid tropism, DNA was extracted from PCLS and the AAV transgene was amplified using PCR. Nanopore sequencing was employed to identify and quantify unique barcodes associated with each capsid. The barcode counts were normalized against the direct input of the AAV library to evaluate and compare individual capsids presence in tumour versus cirrhotic PCLS.

Results: Three hepatectomy specimes carrying HCCs were perfused for 24, 36 and 96 hours. RTCM demonstrated high viability of cirrhotic and HCC samples with RTCM scores ranging between 0-1. RTCM scores correlated well with standard H&E histopathological workup. Nanopore sequencing showed that in cirrhotic PCLS, the observed distribution of AAV capsid counts was heterogeneous, varying between patients. In the HCC PCLS, the distribution was more uniform. One AAV capsid (#45) consistently occurred at higher percentages compared to the other capsid variants. This pattern was observed across all HCC samples from all three patients.

Conclusion: Following perfusion of three cirrhotic HCC livers for up to 96 hours, viability was maintained in both cirrhotic liver tissue and HCC, demonstrating the feasibility of our tumour model. Infection with an AAV library of 49 capsid variants revealed a recurring pattern in HCC samples, identifying AAV capsid #45 as a promising candidate for targeted HCC therapy.

PO5-08-YI
Investigation of GLI1 selective inhibitor for targeted therapy in Intrahepatic Cholangiocarcinoma: a step towards precision medicine

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Background and aims: Intrahepatic cholangiocarcinoma (**iCCA**) is a biliary tract cancer, carrying aggressive nature and poor prognosis, with a 5-year survival rate <20%. The incidence and mortality of iCCA have escalated globally, due to late diagnoses, suboptimal chemotherapy responses, and a scarcity of targeted treatment options. A critical challenge in managing iCCA is its high inter- and intra-tumoral heterogeneity, which complicates biomarker discovery and the development of effective therapeutic strategies, impacting patient outcomes. World Health Organization (*ICD-O-3.2*) has endorsed the differentiation of iCCA into *small* and *large* bile duct types based on its molecular, histological, and clinical features. However, the relationship between molecular characteristics and specific iCCA subtypes remains unexplored, representing a significant gap in translational research. The **Hedgehog** (Hh) pathway plays a fundamental role in the onset and advancement of iCCA, influencing tumor growth, viability, cancer stem cells activation, and epithelial-mesenchymal transition. This research project seeks to assess the effects of a new natural compound known as **Glabrescione B** (GlaB) on Gli1 inhibition in primary and established cell lines *in vitro*, targeting the most important transcription factor and final effector in the Hh pathway.

Method: The response of GlaB has been assessed by Trypan Blue Exclusion test. MTS assay was conducted to ascertain the IC_{50} value. Western blot has been utilized for analyzing the target protein levels. Wound-healing assay has been used to evaluate the ability of cells to migrate. Colony formation assay has been conducted to assess the ability of cancer cells to form colonies. Flow cytometry analysis has been executed to examine cell death induction. The findings were validated in a minimum of three separate experiments and the quantitative data has been presented as the mean±SEM. Student's t-tests were used to evaluate variances between two groups in paired samples. A p-value lower than 0.05 was considered as statistically significant (*p<0.05;**p<0.01;***p<0.001).

Results: Our results show that treating iCCA cells with either free GlaB or HA-GlaB results in a significant decrease in cell proliferation, viability, migration, colony-forming ability, and Gli1 protein levels in a dose- and time-dependent manner (0.05<p<0.001). Moreover, the drug is able to induce iCCA cell death after the administration, compared to the controls.

Conclusion: A new natural compound, called Glabrescione B, has the ability to hinder the growth, survival, invasiveness of iCCA cells and trigger their death. Collectively, this information offers understanding of a novel and possibly advantageous natural chemical compound for treating iCCA *in vivo*.

PO5-16-YI
Impact of inhibiting the endoplasmic reticulum stress response on early carcinogenesis and Epithelial-mesenchymal transition in hepatocellular carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) stand as a major challenge in oncology, mainly due to its late diagnosis and limited treatment options. Several pathways and underlying mechanisms are suggested to promote HCC development. One potential driver of HCC progression is the activation of endoplasmic reticulum (ER) stress pathways. The role of ER-stress in HCC pathogenesis has emerged as a critical area of investigation, because of its involvement in cellular homeostasis and in the unfolded protein response (UPR). ER-stress activation also contributes to early HCC carcinogenesis and facilitates epithelial-mesenchymal transition (EMT), driving tumor formation and progression. This study explores the impact of pharmacologically inhibiting the PERK pathway, a key component of the ER stress response, on early carcinogenesis and EMT in HCC.

Method: A chemically induced mouse model for HCC was used and were treated twice per week with the ER-stress inhibitors TUDCA and/or AMG-PERK for 3 weeks. Liver samples were taken after 25 weeks for histological and molecular biology analyses. The HCC-cell lines (HepG2, Huh7 and SNU449) were used to study several aspects with regards to ER-stress inhibition on early HCC and EMT.

Results: Our findings from this ongoing study reveal that inhibiting the PERK pathway significantly reduces tumor burden and cellular proliferation in a chemically induced HCC mouse model, which was assessed by H&E, PCNA and ki67 histological staining. Collagen deposition levels in mice treated with ER-stress inhibitors were also significantly reduced. Furthermore, Golgi Phosphoprotein 2 (Golph2) antibody has been previously reported to be an indicator for early-stage HCC, as well as a cell proliferation and metastasis biomarker. In this study, Golph2 was found to be decreased after treatment with ER stress inhibitors. HCC-cell lines treated with ER stress inhibitors showed a decrease in tumor cell migration. Moreover, we observed alterations in ER-stress, inflammation, fibrosis, cell proliferation and EMT-related gene expression, leading to a decrease in cell migration and HCC progression *in vivo* and *in vitro*

Conclusion: By using both *in vivo* and *in vitro* models, findings indicate that ER stress inhibitors decrease tumor burden, fibrosis, cell proliferation and metastasis in HCC mouse model. Parallelly, targeting PERK signaling pathway in different cell cultures revealed a modification in ER-stress and EMT expression, leading to a constraint in cell migration. Therefore, this highlights the role of ER stress in HCC pathogenesis and suggest that targeting the PERK signaling pathway may offer a novel therapeutic strategy to impede HCC progression.

PO5-19-YI
miR-30e-3p/CXCL3 axis predicts early tumor escape in sorafenib-treated hepatocellular carcinoma patients

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Background and aims: Curative treatment options for hepatocellular carcinoma (HCC) remain largely limited to a minority of patients identified at early disease stages. Immunotherapy and Tyrosine Kinase Inhibitors (sorafenib and lenvatinib) represent the first-line treatments in advanced cases. Despite immunotherapy has revolutionized HCC treatment, only a minority of patients show a prolonged response. The identification of biomarkers predictive of drug response or early tumor escape remains an unsolved clinical need. MicroRNAs and chemokines are pivotal players in the progression and development of drug resistance in HCC. We previously reported higher miR-30e-3p levels in non-responder patients undergoing sorafenib treatment. Here, we aimed at identifying novel miR-30e-3p targets involved in sorafenib response and, at the same time, representing biomarkers of treatment response.

Method: Serum and tissue miR-30e-3p and CXCL3 levels were analyzed by qPCR analysis in HCC patients and DEN-HCC rats. Functional analysis (qPCR, Western Blot, ELISA) and luciferase reporter assay assessed CXCL3 targeting by miR-30e-3p in transiently transfected and stably infected HCC cell lines. ELISA assay evaluated serum CXCL3 levels in sorafenib-treated HCC patients. Statistical analysis was performed to investigate clinicopathological associations.

Results: CXCL3 resulted upregulated in human and rat HCCs and showed a direct correlation with CXCR2 receptor and a negative one with miR-30e-3p. Functional analysis and luciferase reporter assay demonstrated CXCL3 targeting by miR-30e-3p in HCC cell lines. In the HCC rat model, higher CXCL3 tissue levels correlated with sorafenib resistance, showing a negative correlation with apoptotic markers and tumor suppressor genes and a positive one with tumor size. Before treatment, lower CXCL3 levels associated with microvascular invasion in human HCCs. At two-month follow-up, higher CXCL3 and miR-30e-3p levels were observed in blood samples of non-responder patients. Moreover, CXCL3 levels inversely correlated with days of treatment and positively correlated with neutrophil count whereas miR-30e-3p positively correlated with alfa-fetoprotein. CXCL3 and miR-30e-3p showed a promising predictive potential at the ROC curve analysis.

Conclusion: CXCL3 is a novel miR-30e-3p target in HCC and is involved in sorafenib resistance. If validated in larger cohorts, CXCL3 and miR-30e-3p represent promising circulating biomarkers of early tumor escape in advanced HCCs receiving sorafenib treatment.

PO5-20
Lipid involvement in modeling macrophage phenotype in primary liver cancer

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Background and aims: Primary liver cancer remains a global health challenge and its incidence is growing worldwide. The major histological type is hepatocellular carcinoma (HCC), while intrahepatic cholangiocarcinoma (iCCA) is the second most common primary hepatic malignancy. Lipids play a pivotal role in the pathobiology of cancer by sustaining its onset, progression, and maintenance. Lipids, derived from the tumor microenvironment or synthesized by cancer cells themselves, influence the recruitment, activation and function of immune cells. In particular, lipid accumulation and enhanced fatty acid (FA) oxidation in tumor-associated macrophages contribute to polarization towards an immunosuppressive phenotype that supports tumor development. In this study we investigated whether an altered lipid in iCCA and HCC is associated with modulation of macrophage phenotype.

Method: A liquid chromatography-tandem mass spectrometry analysis was performed to determine the lipid profile of non-tumoral (NT) liver tissue and matched HCC (n=8) or iCCA (n=19) specimens. The macrophage phenotype and lipid content were assessed by flow cytometry. Single-cell RNA sequencing (scRNA-seq) was performed on NT liver- and tumor-infiltrating CD45+ cells obtained from HCC patients (MASLD-associated n=6, viral-associated n=4).

Results: The untargeted lipidomic profile revealed that most classes of lipids were altered in iCCA, showing FA accumulation compared with NT tissue. The influence of FA on macrophage polarization was evaluated by co-culturing primary iCCA cells with the THP-1 monocyte cell line. After 4 hours, the lipid content and the expression of the M2 markers (CD163 and CD11b) were increased in THP-1 cells. In contrast, co-culture of THP-1 cells with normal human cholangiocytes was without effect. Moreover, treatment with palmitic acid increased the lipid content and CD163 expression in THP-1 cells. Lipidome analysis of HCC compared with NT tissue showed a trend towards accumulation of triglycerides and their precursors FAs, mostly in poorly differentiated tumors. Co-culture experiments with primary HCC cells revealed that lipid content in THP-1 increased only after 24h, concomitantly with enhanced expression of the M2 markers CD11b and CD163.Preliminary data of scRNA-seq analysis indicated an enrichment of lipid-related pathways in macrophages.

Conclusion: These results suggest that in the presence of primary tumor cells, monocytes modified their lipid content and acquired an M2 phenotype. FA showed a similar effect. The time lag observed in macrophage polarization towards an M2 phenotype could be due to differences in lipid content between iCCA and HCC.

PO5-21
Clinical relevance in liver cancer patients and cholephilic characteristics of microbial bile acids conjugates

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Background and aims: Upon secretion to the intestine, bile acids (BAs) are transported back to the liver to complete the so-called enterohepatic circulation. It has been recently described that rare microbial BA conjugates (MBACs) are generated during intestinal transit of BAs. Here, we have elucidated whether MBACs behave similarly to major BAs and whether their presence in bile can be associated with hepatobiliary dysfunction.

Method: Three MBACs were chemically synthesized by the formation of an amide bond between cholic acid (CA) and tyrosine (Tyr-CA), phenylalanine (Phe-CA), and leucine (Leu-CA). In patients undergoing surgical or endoscopic bile collection, MBAC levels were determined in bile by HPLC-MS/MS. MBAC cholephilic behavior was evaluated in cellular and animal models.

Results: MBACs were detected (typically <100 molecules/106 total BA) in bile of some (but not all) patients with liver cancer - hepatocellular carcinoma (8/12), and extrahepatic (7/33), and intrahepatic (7/10) cholangiocarcinoma- as well as other malignant - pancreatic ductal adenocarcinoma (27/55) - and non-malignant - benign stenose (4/10), and choledocholithiasis (8/25) - conditions. An inverse relationship with total BA concentrations, but not with age, or outcomes of cancer patients was found. Intravenous injection of GCA and MBACs to rats, resulted in similarly efficient secretion into bile. Adding GCA and MBAC to the microbiota-free in situ isolated rat ileum lumen resulted in similar biliary secretion of intact MBACs. In cells expressing human BA transporters (NTCP, ASBT, OATP1B1, and OATP1B3), MBACs uptake was enhanced, which was hampered by specific inhibitors.

Conclusion: MBACs behave as cholephilic compounds. They are taken up by BA transporters with efficacy similar to that of major primary conjugated BAs. The appearance of MBACs in the bile of some cancer patients is not related to the hepatobiliary dysfunction but probably to changes in the microbiota that favor the proliferation of species able to generate MBACs.

PO5-22
CD147 as a biomarker of hepatocellular carcinoma. Use of AV- CD147 extracellular vesicles as a diagnostic tool

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Background and aims: Extracellular vesicles (EVs) are nano-sized, membranous structures secreted into the extracellular space. They mediate cell-cell communication and carry multiple factors derived from their cell of origin. The lack of reliable biomarkers for Hepatocellular carcinoma (HCC) diagnosis make them good candidates to study in the context of liver cancer. Aim: To study AV-CD147 extracellular vesicles as biomarkers for the diagnosis of hepatocellular carcinoma.

Method: 96 patients were included. Non-cirrhotic liver disease (control group: 8), 38 patients with cirrhosis and 50 with HCC from the Liver Disease department of the Hospital Virgen del Rocío. EVs were characterized by size (0.2-1µm Megamix Plus SC protocol, BD) and the expression of CD147 and phosphatidylserine (the binding partner of annexin V) cell surface by flow cytometry in plasma heparin samples. CD147 gene expression levels were determined in liver biopsy of 25 patients undergoing transplantation/resection (6 cirrhotic, 12 non-cirrhotic patients who developed HCC and 7 cirrhotic patients with HCC). Total mRNA was isolated from tumoral and non-tumoral tissue and gene expression was determined by qPCR.

Results: In our cohort, 90.6% were men, with a mean age of 64.6±9.5 years. Main etiologies were alcohol consumption and HCV infection. AFP, ALT, and GGT levels were significantly increased in HCC group vs. F4 ($p<0.001$, $p=0.05$ and $p=0.02$ respectively). The level of EVs AV+CD147+ U/µl was significantly increased in HCC patients vs. cirrhotic patients (cirrhotic: 1096±211 vs. HCC: 6374±1294 U/µl; $p=0.002$), demonstrating an AUC of 0.82 (0.715-0.915; $p<0.0001$) for HCC diagnosis (spec: 80%; sens: 70.8%, NPV 83.3% and PPV 74.3% (cut off: 1111.21U/µl)). In the multivariate analysis, age, AFP serum levels and AV+CD147+EVs (OR: 12.7 (2.06-78.81) $p=0.006$) were independently associated with HCC diagnosis. Additionally, gene expression levels were significantly higher in tumor tissue compared to non-tumor tissue, regardless of the degree of fibrosis (HCC vs. non-F4: fold-change 3.74 ± 0.53; $p < 0.001$ and HCC vs. F4: 2.08 ± 0.32; $p = 0.004$). No differences were observed in CD147 expression among tumors, independently of liver fibrosis stage.

Conclusion: CD147 expression levels are increased in HCC tumor tissue, independently of liver fibrosis stage. In addition, circulating EVs AV+-CD147+ levels are higher in HCC patients compared to cirrhotic patients, suggesting the potential use of these vesicles as a diagnostic tool for HCC.

PO5-23
MicroRNAs as promising therapeutic inhibitors of liver fibrosis and HCC

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Background and aims: Advanced liver fibrosis and cirrhosis are major risk factors for hepatocellular carcinoma (HCC), a leading cause of cancer related deaths, worldwide. Together, they affect several million people, each year. The dearth of robust anti-fibrotic therapeutic targets, as well as the largely refractory nature of HCC towards most conventional therapies, warrants the need for novel and more effective treatment options. Small, non-coding microRNAs (miRNAs) play regulatory roles in several basic biological processes and are frequently deregulated in multiple disease states including fibrosis and cancer. Our work aims to evaluate and highlight the promising therapeutic potential of miRNAs against liver fibrosis and HCC.

Method: To identify anti-fibrotic miRNAs, we performed functional screens using patient-derived primary human hepatic myofibroblasts, the key drivers of fibrosis, followed by *in vivo* validation in mouse models of fibrosis. MiRNAs with potential tumor suppressor and oncogenic roles in HCC were identified using microRNA expression profiling of liver tumors from conditional oncogene-driven mouse model of HCC at different stages of tumor development and regression. Extensive downstream *in vitro* and *in vivo* analyses in three independent mouse models of HCC to determine the effect of their modulation on HCC was done.

Results: In our fibrosis study we discovered miR-190b-5p and miR-296-3p as two novel robust anti-fibrotic miRNAs, which we found were significantly reduced in human livers with fibrosis. Importantly, we showed an anti-fibrotic function for both miRNAs in a human liver bud model. In our HCC studies, we found that different stages of HCC development and regression show distinct miRNA profiles. We identified and validated miR-342-3p and miR-20a-5p as potent tumor suppressor and oncogenic miRNAs, respectively. Therapeutic modulation of these microRNAs, significantly attenuated HCC development and prolonged survival in tumor-bearing mouse models of HCC.

Conclusion: In our fibrosis study we discovered miR-190b-5p and miR-296-3p as two novel anti-fibrotic miRNAs. Our HCC studies show global miRNA expression distinctly changes as liver tumors develop and as they regress. We also show that miRNAs are effective against HCC leading to attenuation of tumor development and prolonging the survival of liver tumor-bearing mouse models of HCC. Together, our work highlights the promising therapeutic efficacy and potential clinical utility of miRNA modulation in the treatment of liver fibrosis and HCC - disease states with significant global impact.

PO6-02
Mitochondrial stress in patients with chronic hepatitis B-related hepatocellular carcinoma

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Background and aims: Hepatitis B virus (HBV) interacts with hepatic mitochondria and increases liver oxidative stress. We postulated that HBV-induced mitochondrial stress might account for hepatocellular carcinoma (HCC) development in patients with chronic hepatitis B (CHB).

Method: Fifty (50) mono-infected patients with CHB and HCC and 22 healthy controls were included in the study. Liver mitochondrial DNA (mtDNA) damage was screened by long PCR, Southern blot and Slot blot analysis. The expression of the mtDNA-encoded cytochrome *c* oxidase subunits 1 (MT-CO1), Parkinson-juvenile disease protein 2 (PRKN), Phosphatase and Tensin-Induced Putative Kinase-1 (PINK1), Lon Peptidase 1(LONP1), mitochondrial chaperonins HSPD1 and HSPA9, as well as liver pro-inflammatory cytokines TNF-alpha and IL6 mRNA was investigated by Western-blotting and/or Reverse Transcriptase-quantitative PCR.

Results: Whereas 100% of patients with HCC exhibited multiple mtDNA deletions, only 12% of the controls of comparable age (κc^2=6.6; p<0.01) carried a single common mtDNA deletion. Significant decreases were observed in patients with HCC as compared to controls for the mtDNA-encoded MT-CO1 (0.69 ± 0.28 and 1.32 ± 0.88, *p<0.001*), nuclear DNA-encoded HSPA9 (0.44 ± 0.21 and 1.11 ± 0.19, *p<0.001*), HSPD1 (0.82 ± 0.25 and 1.79 ± 0.63, *p<0.05*), LONP1 (0.80 ± 0.17 and 1.12 ± 0.34, *p<0.05*), PRKN (0.44 ± 0.28 and 1.09 ± 0.41, *p<0.0001*), and PINK1 (0.52 ± 0.06 and 1.12 ± 0.25, *p<0.0001*). Liver TNFalpha mRNA was 1.53 ± 0.2 and 0.84 ± 0.16 (*p<0.05*) and IL6 mRNA was 6.98 ± 0.88 and 1.25 ± 0.32 (*p<0.05*) in patients with HCC and controls, respectively. Protein levels of MT-CO1, LONP1, PRKN, and PINK1 significantly decreased in patients HCC compared to the healthy controls.

Conclusion: Diverse mtDNA damages were associated with alterations in mitochondrial function, mitochondrial unfolded protein response, mitophagy and liver inflammation in patients with CHB and HCC.

PO6-03
Identification of Novel Extracellular VEsicle microRNA signatures in HepatoCellular Carcinoma patients with different cirrhotic aetiology (NEVER-HCC)

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Background and aims: Hepatocellular carcinoma (HCC) is a primary malignancy of the liver generally associated with cirrhosis of various etiologies (viral and non viral). Extracellular vesicles (EVs), particularly exosomes, carry microRNAs (miRNAs) that reflect the physiological or pathological state of their origin. Identifying specific EV-miRNA signatures associated with HCC in patients with different cirrhotic backgrounds could offer new insights into the mechanisms of tumorigenesis and potential diagnostic biomarkers.

This study aims to identify and characterize novel EV-miRNA signatures in HCC patients with distinct cirrhotic etiologies to enhance the understanding of tumor biology and improve diagnostic precision.

Method: EVs were isolated from plasma samples of HCC patients with different cirrhotic etiologies (N=33) and patients without HCC (N=34). High-throughput sequencing was conducted to profile the miRNA content in these EVs. Bioinformatic analyses were performed to identify differentially expressed miRNAs specific to each cirrhotic etiology, and potential pathways associated with these miRNAs were investigated.

Results: We identified unique EV-miRNA signatures for each cirrhotic etiology associated with HCC. For instance, specific miRNAs (e.g., miR-21, miR-122) were predominantly found in patients with viral cirrhosis, whereas other miRNAs (e.g., miR-155, miR-192) were identified in patients with non viral cirrhosis (alcoholic and MASLD-related cirrhosis). Pathway analysis revealed that these miRNAs are involved in regulatory networks related to liver fibrosis, inflammation, and cellular proliferation, which are critical in HCC pathogenesis.

Conclusion: The identification of etiology-specific EV-miRNA signatures in HCC patients offers potential biomarkers for early diagnosis and insights into the pathophysiological mechanisms underlying HCC development in the context of different types of liver cirrhosis. Further validation studies are necessary to confirm the clinical applicability of these miRNA signatures in non-invasive HCC diagnosis and personalized patient management.

PO6-06
Novel prooncogenic mechanism of ABCC3/B3H7 in liver metastasis using a hepatocellular cancer model

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Background and aims: Hepatocellular cancer (HCC) is the malignancy responsible for a large number of cancer deaths worldwide as a consequence of drug resistance, metastasis, and tumor recurrence. ABCC3, a multidrug-resistant protein, has been implicated in liver carcinogenesis and is associated with poor prognosis when overexpressed. B3H7, an immunoregulatory protein, has recently been identified as a potential promoter of tumor invasion through its regulation of epithelial-mesenchymal transition in vitro. Until now, the role that both proteins may exert in tumor metastasis is unknown. This study aimed to demonstrate whether ABCC3/B3H7 participates as tumor metastasis promoter molecules.

Method: We used the parabiosis model, which united a rat with chemical carcinogenesis and a control rat.

Results: The chemical carcinogenesis model demonstrated the coexistence of ABCC3/B3H7 in the tumor area. Interestingly, in the control rats of the parabiosis model for each carcinogenic stage, protein levels of both proteins were overexpressed in the bloodstream and the liver, showing the coexistence of ABCC3/B3H7 positive cells. Furthermore, to confirm the immunoregulatory role of B3H7, the infiltrate of CD4+ T and CD8+ T lymphocytes found in the tumor tissue was evaluated.

Conclusion: Our study's findings are significant as they demonstrate the pro-oncogenic role of ABCC3/B3H7 in promoting metastasis and tumor recurrence. This suggests that targeting ABCC3/B3H7 could be a promising therapeutic strategy, particularly in the context of liver transplantation. For instance, developing drugs that inhibit the overexpression of ABCC3/B3H7 could potentially prevent tumor recurrence or metastasis, improving the outcomes of liver cancer patients.

PO7-02
Immune biomarkers and their network of connections in patients with hepatocellular carcinoma and liver cirrhosis associated with hepatitis C virus

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Background and aims: Hepatocellular carcinoma (HCC) is associated with tumor- and host-mediated immune response imbalance. Sustained virological response (SVR) reduces but does not eliminate the risk of HCC. This study analyzed peripheral soluble biomarkers in patients with HCC with hepatitis C virus (HCV) and with SVR.

Method: 62 cirrhotic adults with HCC were categorized into two groups: viremic (HCC-HCV, n=30) or with SVR (HCC-SVR, n=32). 32 Healthy participants were controls (CT). Comparative qualitative and quantitative parameters (Luminex Bio-Plex), correlations (Spearman), interactions (Cytoscape Consortium 2.8 software), heat maps and visual typology diagrams of peripheral soluble mediators (pro-inflammatory and regulatory cytokines, chemokines, and growth factors) were analyzed by appropriate statistical methods and area under the ROC curve (AUC-ROC).

Results: A significant increase in soluble mediators was observed in the CHC groups compared to the CT group. There was less quantification of CCL3, CXCL10, IFN-g, IL-1Ra, IL-10 and VEGF in the CHC-RVS group compared to CHC-HCV. CXCL10 and CCL3 were more accurate in discriminating HCC groups (AUC-ROC: 0.77 and 0.73, respectively). CXCL10 and CCL3 values lower than 38.6pg/mL and 1.3pg/mL (likelihood ratio of 3 and 15), respectively, qualified the HCV-SVR group. Strong correlations of the integrative network of mediators were observed in the CT, with a significant loss of connectivity in the HCC-HCV group, with an axis between chemokines and pro-inflammatory cytokines, and a slight recovery of connectivity in the HCC-SVR group, with an axis shifted to regulatory cytokines and growth factors.

Conclusion: CXCL10 and CCL3 performed better to characterize HCC groups. The integrative networks of connections between soluble mediators showed distinct profiles between viremic HCC-HCV and with HCC-SVR participants. The greater network of connections in the CHC-SVR group suggests the impact of HCV eradication on immune reconstitution, despite remaining less robust than that observed in the CT group. These results add knowledge about the distinct immune response profiles in patients with viremic HCC or with SVR, which may have clinically significant oncogenic implications.

PO7-03-YI
Oncostatin M modulates the biology of cholangiocarcinoma cells and the tumor microenvironment

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Background and aims: Cholangiocarcinoma (CCA) is a highly aggressive tumor characterized by high resistance to chemotherapy and poor prognosis. Increasing evidence highlights that oncostatin M (OSM) regulates the tumor microenvironment (TME) and orchestrates the crosstalk between cancer and stromal cells. Particularly, OSM-mediated signalling pathways (mainly mediated by p-STAT3) have been associated with proliferation, angiogenesis, and metastasis as well as epithelial-mesenchymal transition (EMT) and abundance of cancer stem cells (CSCs). Since the role of OSM in iCCA has been only partially investigated, this project aims at elucidating the involvement of this factor and its receptor in iCCA progression, as well as in tumor-stroma interaction.

Method: Expression of OSM and its receptor was analysed in patients with iCCA by immunohistochemistry or RT-PCR. Two human iCCA cell lines (HuCCT-1 and CCLP-1) and two type of cultured stromal cells have been used in this study. Cell migration and invasiveness of iCCA cells has been evaluated by performing chemotaxis and invasion assays. Protein expression and activation of signalling pathways was performed by Western blotting. Knockdown of OSMR and gp130 was carried out with specific siRNA in iCCA cells. The interaction between iCCA and stromal cells was evaluated treating hepatic stellate cells, HSCs, and cancer-associated fibroblasts, CAFs with conditioned medium collected from iCCA cells.

Results: iCCA cells expressed both OSM receptors and OSM at protein levels. In human CCA specimens, OSM was expressed at higher levels in cancer cells and in the tumor microenvironment with respect to peritumoral tissue. In addition, OSMR mRNA levels were higher in CCA. Exposure of iCCA to OSM induced a dose-dependent increase in cell migration and invasion. These effects were likely mediated by cytoskeletal rearrangement, as indicated by increased expression of p-FAK, p-paxillin and p-MLC2 and inducing EMT. OSM also upregulated cancer-associated pathways including c-Myc, G6PD, p-Rb, and p-Akt The ability of OSM to induce iCCA cell migration and invasion was reduced after knockdown of the OSMR or of gp130, which transduces its signal downstream. Moreover, preincubation with ruxolitinib, a pharmacologic inhibitor of Jak2, blocked the biologic effects of OSM. Incubation of primary HSCs or CAFs with conditioned medium collected from iCCA cells treated with OSM resulted in increased cell migration, suggesting a role in the formation of a dense fibrotic tumor microenvironment.

Conclusion: This study identifies the OSM/OSMR axis as a novel system potentially implicated in cholangiocarcinogenesis with pro-tumorigenic effects and modulation of the tumor microenvironment.

PO7-08
Combinatorial effect of sorafenib, valproic acid and metformin in a fluorescent xenotransplant of hepatocellular carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) is the main type of primary liver cancer. Worldwide, ocuppies the sixth and fourth place in incidence and mortality, respectively, with a 1:1 morbidity/mortality ratio. Systemic treatment for advanced HCC is based on sorafenib, it extends the overall survival in three months. Beside this, latest research with drugs used in diseases other than cancer, like the histone deacetylases inhibitors or rapalogues, have been described with *in vitro* and *in vivo* anticancer properties and they have shown synergistic effects in combination with approved anticancer drugs. Combined schemes have increased the apoptosis induction, inhibition of angiogenesis, inhibition of migration and inhibition of proliferation. In this study, we evaluated the viability, proliferation, migration, clonogenicity and angiogenic potential using a combination of sorafenib, valproic acid and metformin in a cellular model of HCC.

Method: HepG2 cells were treated with different concentrations and combinations of sorafenib, valproic acid and metformin to evaluate viability and proliferation with CellTitter-Blue reagent. Hepa1 and normal human dermal fibroblast were treated with drug combination and viability evaluated with the former reagent. Migration was assessed by a wound healing assay, angiogenic potential was evaluated with qPCR with probes spanning on vascular endothelial growth factor A (*VEGF-A*) and glyceraldehyde phosphate dehydrogenase *(GAPDH)* genes. Reproductive cell death was determined by clonogenic assays. Cell death was evaluated by flow cytometry using anexinV/propidium iodide as markers. Caspase 9, 3, poly ADP-ribose polimerase 1 (PARP), p62, LC3AB and GAPDH were detected by immunoblotting.

Results: HepG2 cells exposed to sorafenib, valproic acid or metformin showed a reduced viability and proliferation in a dose-dependent manner. Drug combination showed the strongest viability reduction with consistent results across HCC cell lines. Also normal cells viability was less affected. Drug combination reduced wound closure and colonies but did not modulate *VEGF-A* mRNA. Cell death was increased in drug combination without activation of caspase 9, 3 or PARP, together with accumulation of p62 without LC3 conjugation.

Conclusion: Single administration of sorafenib, valproic acid and metformin reduced viability and proliferation of HepG2 cells in a dose-dependent manner. Drug combination has the most synergistic effects with consistent result across different HCC cell lines and a lesser effect in normal cells. Migration and clonogenicity were decreased as a result of drug combination. Drug combination induced cell death without involvement of apoptotic related caspases. Sorafenib increased autophagic related proteins in respect to drug combination who impaired this survival process.

PO7-09-YI
Transarterial embolization with ethylene vinylalcol copolymer (EVOH) for spontaneous rupture of unresectable hepatocellular carcinoma: an oncological role beyond haemostasis?

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Background and aims: Ruptured hepatocellular carcinoma (rHCC) is a lethal complication of HCC. Transcatheter arterial embolization (TAE) is an established treatment for ruptured HCC in the emergency setting, especially when surgery is not an option. TAE with non-adhesive EVOH-based embolic agents has efficacy, feasibility and safety comparable to TAE performed with other embolic agents for abdominal bleeding. We observed the effect of EVOH embolization in patients with rHCC.

Method: 8 patients with spontaneous rupture of unresectable HCC (6 female, 2 male) underwent TAE with EVOH in an emergency setting. Technical and clinical success was evaluated. Complications, bleeding control and 30-day mortality were recorded. Tumour volume reduction at 30 and 90 days after treatment was also evaluated with Contrast-Enhanced Computed Tomography (CE-CT). Residual Vital Tumor (RVT) was defined as intralesional enhanced tissue at CE-CT.

Results: The procedure was successful in all patients. No complications were reported. The mean operative time was 98.125 minutes. 30-day mortality was 0%. The mean pretreatment lesion volume was 687.82 cc. At 1 month follow-up after TAE, the mean lesion reduction was 645.57. At 3 months the mean reduction was 510.78 cc with an RVT of 421.57 (6 patients, one missing). During follow-up, 4 patients were eligible for surgery as a result of treatment, 2 of whom underwent epatectomy.

Conclusion: In this first reported case series, TAE with EVOH was effective in the treatment of bleeding from ruptured HCC. Beyond emergency treatment, post-procedural evaluation of TAE showed an oncological effect with tumour downstaging, making some patients eligible for surgery.

PO7-13
Alcohol etiology: a key factor in the management of hepatocellular carcinoma

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Background and aims: In France, 70 to 85% of Hepatocellular Carcinoma (HCC) are the result of liver disease related to chronic alcohol consumption. Sorafenib, a tyrosine kinase inhibitor (TKI), was the only approved treatment for advanced HCC from 2007 to 2021. Resistance to treatments, particularly sorafenib, is frequently reported. Here, we explored the consequences of chronic alcohol exposure on the response to sorafenib. Currently, the etiology of hepatocellular carcinoma is not considered in the treatment choice. First, we determined the response to sorafenib in our chronic alcohol exposure (CAE) cell model. Then, we conducted a retrospective study on the CHIEF cohort (Amiens University Hospital) of patients with hepatocellular carcinoma treated with sorafenib.

Method: We used an HCC cell line:Huh-7 cells. They were exposed for more than 6 months to alcohol. First, using an MTT viability test, we evaluated the in vitro response to sorafenib by the Inhibitory Concentration 50 (IC50). The signaling pathways involved in sorafenib resistance were studied by western blot. Secondly, the response to sorafenib was assessed in a retrospective study of 86 HCC patients from the CHIEF cohort, using RECIST 1.1 criteria. We categorized the patients into two groups, HCC of alcoholic etiology and HCC of non-alcohol etiology (HCV, HBV, MASH, and others). Overall survival and progression-free survival of the weighted groups are compared using the Logrank test.

Results: Our cell model results show that CAE significantly decreases cell sensitivity to sorafenib ($p = 0.006$), indicating increased resistance due to alcohol. Preliminary studies suggest that the ERK pathway plays a role in sorafenib resistance. In our clinical study, patients with alcohol-related HCC are less responsive to sorafenib than patients with HCC of other etiologies (35% responders vs. 65%). Moreover, the overall survival is significantly different between the two groups ($p = 0.0447$). The median survival is 9.5 months and 12.1 months in the 'Alcohol' and 'Other etiologies' groups, respectively. Progression-free survival is also significantly lower, 5.68 and 9.66, in the alcohol group compared to the other etiologies group ($p = 0.489$).

Conclusion: Using our cell model, we demonstrated that CAE induces resistance to sorafenib. Our retrospective study shows that sorafenib is less effective in patients with alcoholic HCC than in patients with non-alcoholic HCC. The induction of resistance to sorafenib by alcohol leads us to believe that it might not be effective for treating patients with alcohol-related HCC.

PO8-06-YI
Liquid Crystalline Nanoparticles based delivery of an Anticancer bioactive, Methotrexate

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Background and aims: Liver cancer is a disease of uncontrolled cell growth, which may invade adjacent tissue and cause infiltration beyond the liver. Most of the potent and effective anticancer drugs used in liver cancer therapy shows poor bioavailability at desired site as well as toxic in nature. The aim of the study was to investigate mannose modified Liquid Crystalline Nanoparticle (LCNPs) carrier for efficient and site specific delivery of potent anticancer drug (Methotrexate) used in hepatic carcinoma therapy.

Method: MTX loaded LCNPs were prepared by lipid cast film method and sonication method. The nanoparticles were characterized *in-vitro* for their shape, size, percent drug entrapment and stability by Optical Microscopy, Cross Polarized Light Microscpy (CPLM), Transmission Electron Microscopy (TEM), X-ray diffraction (XRD) and Atomic Force Microscopy (AFM).

Results: *In-vitro* stability studies reveal that LCNPs formulations are stable for 120 days at room temperature. *Ex-vivo* cell cytotoxicity was performed on Human hepatoma cell line. *In-vivo* studies included fluorescence microscopy and organ distribution studies which show the Mannose modified LCNPs exhibit better accumulation in liver as compared to unmodified system. The results of the present study indicate, this system is more stable as compared to other system.

Conclusion: Eventually it may be concluded that incorporation of MTX in mannose modified LCNPs increases the residing time of drug in the body by altering of pharmacokinetics and biodistribution pattern, and the drug primarily concentrates in the liver. This system showed excellent cytotoxicity towards cancer cells. From the present investigation it is evident that this system may be used for liver cancer and other liver disease.

PO8-09-YI
Data Mining Reveals Novel Gene Drivers of Lenvatinib Resistance in Hepatocellular Carcinoma

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Background and aims: Liver cancer is the sixth most common malignancy and the third leading cause of cancer-related deaths globally. Hepatocellular carcinoma (HCC) is the most prevalent type, accounting for nearly 90% of all liver cancer cases. The first-line systemic therapy for advanced HCC includes lenvatinib, an oral multi-kinase tyrosine inhibitor. However, many HCC patients exhibit resistance to lenvatinib, leading to treatment failure. Recent studies suggest that lenvatinib resistance is multi-factorial.

Method: Four public RNA-seq datasets were retrieved from Gene Expression Omnibus database and further analysed to identify novel gene drivers of lenvatinib resistance.

Results: After applying several filtering conditions, Gene Ontology (GO) and pathway enrichment analyses using Kyoto Encyclopaedia of Genes and Genome (KEGG) databases to identify significantly enriched pathways, a total of five genes emerged as good candidate genes which are likely to be associated with lenvatinib resistance: *SEZ6L2, SECTM1, FBLN7, IFI6,* and *NPC1L1.* The association between these five genes with patient's prognosis was based on TCGA database. Our validation using Huh7 and Hep3B HCC cells treated with lenvatinib showed increased consistent mRNA expressions of *SECTM1* and *IFI6* after 48 hours of treatment.

Conclusion: Further research is needed to clarify the molecular pathways involving these genes that may enable HCC tumors to develop lenvatinib resistance.

PO8-20
Evaluation of the effect of 3'5-dimaleamylbenzoic acid on preneoplastic lesions in a model of chemical hepatocarcinogenesis

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Background and aims: Hepatocellular carcinoma (HCC) is the most common malignant neoplasm of the liver, with an alarmingly high mortality. Metabolic reprogramming, increased glutathione concentration, and activity due to the high requirement of energy and biomolecules to promote cell proliferation are some of the main modifications in tumor cells. In this sense, prooxidants that target glutathione depletion are part of the new approaches to chemotherapeutics. Previously, 3′5-dimaleamylbenzoic acid (3'5-DMBA) has been shown to decrease intracellular glutathione levels. Our objective was to investigate the effects of 3'5-DMBA administration on histopathological alterations and preneoplastic lesions in the modified model of the resistant hepatocyte.

Method: Histopathological analysis was performed using hematoxylin and eosin staining, Masson's trichrome, and Schiff's periodic acid staining. The expression of proinflammatory and oxidative stress markers was assessed by immunohistochemistry.

Results: Treatment with 3'5-DMBA attenuated histological alterations, decreased glycogen and collagen accumulation, and reduced the number and size of preneoplastic lesions. Additionally, the expression of the inflammatory markers cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and the lipid peroxidation marker 4-hydroxynonenal (4-HNE) were attenuated by this treatment.
Conclusion: our results show the potential therapeutic effect of 3'5-DMBA, a prooxidant, for treating HCC.

PO8-23
Genetic variants in lipid metabolism and inflammation for HCC risk stratification and prognostication in MASLD

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Background and aims: Metabolic dysfunction-associated steatohepatitis (MASH) represents a severe form of non-metabolic-associated fatty liver disease (MASLD), which can progress to cirrhosis and hepatocellular carcinoma (HCC). The progression of MASH to HCC is partially influenced by genetic susceptibility. The aim of this study was to assess the combined impact of genetic variants in genes involved in lipid metabolism and inflammation on the progression of liver damage in a prospective cohort of MASLD patients.
Method: Patients: 181 patients were enrolled in this retrospective longitudinal study. Patients were admitted for MASLD evaluation between January 1, 2004 and December 31, 2018.
Genotyping: Eleven SNPs were genotyped by allelic discrimination.
Statistical analysis: A genetic risk score for HCC was developed from the twelve preselected SNPs. The final model was determined in a multivariate analysis by entering all SNPs for which at least one allele was associated with HCC. This score was estimated by a linear combination of predictors, by using coefficients from the multivariate model. Then, the HCC risk groups were defined from the deciles of this GRS.

Results: MASLD patients (71 with F3-F4 fibrosis) were monitored for a median period of 135 months. During follow-up, 22 patients developed HCC and 33 patients died. Our investigation focused on the genotypes implicated in lipid metabolism (*PNPLA3*, TM6SF2, *MBOAT7*, *HSD17β13*) and inflammation (*Il-1β, Il-6, TNF-α, CCL5-RANTES*).
Multivariate analysis highlight that the *TM6SF2* rs58542926-TT genotype (p = 0.002), *PNPLA3* rs738409-CC (p = 0.034), *TNF-α* rs1800629-AA (p < 0.001) and *TNF-α* rs1800630-AA (p = 0.008) were associated with HCC occurrence.
A genetic risk score based on these 4 SNPs was constructed, enabling the stratification of patients into three groups with a progressive risk of HCC (compared to low-risk group: medium-risk group (HR = 6.71 [95% CI 1.45–31.13], p = 0.015); high-risk group (HR = 143.84 [95% CI 25.49–811.87], p < 0.001) and overall mortality (compared to low-risk group: medium-risk group (HR = 2.57 [95% CI 1.06–6.24], p <

0.001); high-risk group (HR = 27.95 [95% CI 9.95–78.52], $p < 0.001$). Similar results were observed when restricted to patients with advanced liver fibrosis.

Conclusion:
Our findings highlight that SNPs involved in the regulation of lipid turnover and inflammation exert a combined influence on the progression of liver damage in patients with MASLD and may useful for HCC risk stratification regardless of fibrosis status.

PO9-09
Incidence and prevalence of hepatocellular carcinoma in patients coinfected with HBV and HDV

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Background and aims: Delta hepatitis (HDV), an often under-recognized condition, has garnered renewed interest due to new, effective treatments. Despite numerous studies, research has been hindered by small sample sizes, data variability, and inconsistent endpoints. This study investigates the impact of HDV on chronic hepatitis B (HBV) and HBV-related liver cirrhosis, focusing on the progression of liver disease in anti-HDV positive patients over time.

Methods: A retrospective cohort of 44 patients with HDV infection, followed at the Gastroenterology Unit of Policlinico Umberto I, was analyzed. Inclusion criteria were anti-HDV positivity, HBsAg or HBV-DNA positivity, age >18, and a follow-up of at least 12 months. The cohort was compared to a control group of 44 anti-HDV negative patients with similar baseline characteristics. Follow-up started at chronic hepatitis or HBV-related cirrhosis diagnosis and ended at death, liver transplant, or last observation. The HBV group had an average follow-up of 100.4 months, whereas the HDV group had an average follow-up of 104.3 months. Various clinical, laboratory, and imaging parameters were used to assess liver disease progression.

Results: The study confirmed that HDV infection worsens the prognosis of HBV infection. Anti-HDV positive patients showed higher rates of liver cirrhosis, decompensated cirrhosis, complications, hepatocellular carcinoma (HCC), and mortality. The survival rate was significantly lower in the anti-HDV positive group (45.85% at 330 months) compared to anti-HDV negative patients (91.09%). Furthermore, anti-HDV positive patients exhibited worsening clinical, laboratory, and imaging parameters over time. Notably, there was a higher prevalence and incidenze of HCC in anti-HDV positive patients at both the baseline and last observation.

Conclusion: This study highlights the severe impact of HDV on the progression of HBV-related liver disease, showing increased rates of cirrhosis, complications, HCC, and mortality despite similar levels of HBV activity and comorbidities. These findings are consistent with existing literature, underlining the need for revised screening policies for HDV and the expansion of local laboratories capable of performing HDV-RNA tests. Further longitudinal studies with larger sample sizes are needed to better understand the natural history of Delta hepatitis and its management.

PO9-11-YI

The hepatocyte nuclear factor 1 homeobox A (HNF1A) gene polymorphism and AFP serum levels in Egyptian HCC patients

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Background and aims: Hepatocyte nuclear factors (HNF) were first identified as liver-enriched transcription factors that might participate in various activities related to the transcription of genes unique to the liver. The study aimed to reveal the impact of HNF1A gene variations on disease progression in hepatocellular carcinoma (HCC) patients and its relation to serum alpha-fetoprotein (AFP) level.

Method: Participants in the study were classified as Group I, (32 HCC patients), Group II (36 chronic hepatitis C (HCV) patients) and Group III, (26 healthy volunteers) as a control group.
Each patient underwent: full history taking, thorough clinical examination and radiological examination. Furthermore, tumour staging was done using BCLC staging system. HNF1A gene polymorphisms (rs-2464196 and rs-1169310) were genotyped by real-time PCR.

Results: The findings revealed the highest frequency of AA and GA genotypes of HNF1A (rs2464196) polymorphism in both HCC (P= 0.002) and chronic HCV (P= 0.004) patients in comparison with controls. Regarding rs1169310gene polymorphism, no significant variation was observed across various genotypes when comparing the experimental groups to the control group. Additionally, HCC patients harboring the AA genotype for rs2464196 had significantly increased AFP (≥ 200 ng/ml) levels, whereas HCC patients with rs1169310 SNPs for HNF1A had no significant association regarding the AFP level.

Conclusion: The rs2464196 polymorphism of HNF1 is associated with increased AFP levels and HCC disease progression, which may be a prognostic and diagnostic genetic indicator.

POSTER ABSTRACT PRESENTATIONS

Clinical Science

PO2-06-YI
A new prognostic index for patients with advanced biliary tract cancer treated with cisplatin, gemcitabine and durvalumab

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Background and aims: This study aims to identify a new prognostic index for patients with biliary tract cancer (BTC) treated with cisplatin, gemcitabine and durvalumab (CGD) in first-line setting.

Method: The study population consisted of 319 patients with BTC from 11 Eastern and Western Countries. Using multivariate analysis, we previously developed a prognostic model called the CGD index by combining the 5 baseline positive variables and assigning a weight from 1 to 5 as follows: 1 for metastatic disease, 2 for carcinoembryonic antigen increased levels, 3 for albumin decreased levels, 4 for gamma glutamyl transferase increased levels, 5 for neutrophil-lymphocyte ratio ≥ 3. Patients were stratified into three risk-groups as follows: low risk-group (score from 0 to 5), intermediate risk-group (score from 6 to 10), and high risk-group (score from 11 to 15).

Results: Median progression-free survival was 10.5 months [95% confidence interval (CI): 8.4-11.9 months] in low risk-group (27.3%), 8.7 months (95% CI: 7.1-9.9 months) in intermediate risk-group (38.9%), and 5.5 months (95% CI: 4.4-7.4 months) in high risk-group [33.8%; low risk hazard ratio (HR): 0.44, intermediate risk HR: 0.63, high risk HR: 1, $p < 0.01$]. Median overall survival (OS) was 17.9 months (95% CI: 13.5-17.9 months) in low risk-group,15.6 months (95% CI: 10.2-18.4 months) in intermediate risk-group, and 8.0 months (95% CI: 7.4-12.5 months) in high risk-group (low risk HR: 0.32, intermediate risk HR: 0.52, high risk HR: 1, $p < 0.01$). There was no difference in overall response rate (low risk: 28.7%, intermediate risk: 36.3%, and high risk: 29.6%; $p = 0.26$), while disease control rate was significantly different in the three risk-groups (low risk: 78.2%, intermediate risk: 72.6%, and high risk: 61.1%; $p < 0.01$) as well as the rate of patients receiving a second-line therapy (low risk: 21.8%, intermediate risk: 23.4%, and high risk: 17.6%; $p = 0.02$). The safety profile was similar in the three risk-groups, except for nausea (low risk: 36.8%, intermediate risk: 42.7%, high risk: 26.8%; $p = 0.04$), leukopenia (low risk: 28.7%, intermediate risk: 33.1%, high risk: 16.7%; $p = 0.01$), and neutropenia (low risk: 55.2%, intermediate risk: 55.6%, high risk: 23.1%; $p < 0.01$).

Conclusion: The CGD index is an easy-to-use tool able to stratify patients with BTC undergoing first-line therapy with CGD. Further studies are needed to prospectively test and validate this index.

PO2-12-YI
Impact of molecular alterations on outcomes of advanced biliary tract cancers treated with cisplatin, gemcitabine, and durvalumab: a multicenter, real-world study across Europe, Asia, and America

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Background and aims: The TOPAZ-1 trial demonstrated a survival advantage from adding durvalumab to gemcitabine and cisplatin (CGD) for advanced biliary tract cancers (aBTCs). However, evidence on response biomarkers to CGD and clinical impact of genomic alterations remains limited. This study investigates the prognostic impact of genetic alterations on outcomes in aBTCs treated with CGD in clinical practice.

Method: N=513 patients with unresectable, locally-advanced, or metastatic BTC treated with 1st-line CGD at 39 sites across 11 countries (in Europe, USA, Asia) were retrospectively analyzed for baseline characteristics and tumor molecular data from local NGS reports. The primary endpoint was the impact of commonly mutated genes (>3%) on OS/PFS, with secondary endpoints including their impact on disease-control rate (DCR) and survival by disease location. The CGD cohort was compared to a historical cohort receiving 1st-line CG only, and interaction test conducted to identify molecular response predictors.

Results: Most patients were <70 years (56.9%), male (53.9%), with intrahepatic cholangiocarcinoma (iCCA, 55.7%) and metastatic disease (77.3%). Median (m)PFS was 8.4 months and mOS was 15.9 months. In the entire population, multivariate analyses identified SMAD4 mutation as positive prognostic marker for PFS (HR 0.49; p=0.018) and OS (HR 0.11; p=0.023); TP53 mutation negatively impacted PFS (HR 1.62; p=0.0047), while TERT mutation negatively affected OS (HR 8.92; p=0.0012); patients with TP53 and TERT mutations had also inferior DCRs than wild-type counterparts (53.8% vs. 80.1% and 71.0% vs. 81.8%). The interaction test highlighted SMAD4 mutation as positive predictive factor for OS and PFS of CGD.
Subanalyses by disease location showed TP53 mutation negatively affected PFS and OS in iCCA, KRAS mutation was associated with shorter PFS in extrahepatic CCA, while no significant genomic marker was identified in gallbladder cancer, possibly due to limited sample size.
A prognostic model incorporating four prognostic variables identified at multivariate analyses (SMAD4 status, TERT status, disease stage, and ECOG-PS) stratified patients into four risk groups with significant differences in OS (mOS not reached–20.5–11.2–3.6 months, p<0.001) and PFS (mPFS 13.3–9.2–7.1–2.7 months, p<0.001).

Conclusion: This study provides first evidence of the prognostic impact of specific molecular alterations (SMAD4, TP53, and TERT) in a large cohort of CGD-treated aBTC patients. Our prognostic model effectively stratifies patients by risk, highlighting its potential utility, if validated, in guiding personalized treatment. These findings underscore the value of molecular profiling to refine aBTC stratification and optimize therapeutic approaches in clinical practice.

PO2-13

Final safety and efficacy results from the phase 1b/2a study of fostrox plus lenvatinib in second/third line patients with advanced hepatocellular carcinoma who progressed on immunotherapy

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Background and aims: Immunotherapy combination regimens are standard of care as first line treatment of advanced hepatocellular carcinoma (HCC). However, most patients progress within 7 months and there is a high unmet need for effective second line treatments. HCC progression occurs mainly in the liver, in patients with chronic liver disease. Consequently, it is important that novel therapies reduce liver tumor burden without adversely affecting synthetic liver function.

Fostrox is an oral liver targeted nucleotide prodrug, that maximises exposure in the liver by first-pass metabolism. Combining fostrox with Lenvatinib (LEN) exploits potential anti-angiogenic synergy with LEN and targets both intra- and extra-hepatic disease (NCT03781934).

Method: A phase 1b/2a open-label, multi-center, dose escalation/expansion study with fostrox QD (orally, 5 days in 21-day cycles) plus LEN QD (orally, standard doses) at 10 sites in Spain, Korea and UK. Eligibility included: locally advanced unresectable or metastatic HCC, Child-Pugh A, ≤ 2 prior lines of systemic therapy, ECOG PS ≤ 1. Primary objective was to establish RP2D by evaluating safety, tolerability and preliminary efficacy. At final analysis, median follow-up was 10.5 months.

Results: 21 patients were enrolled at fostrox doses of 20mg (3 pts) and 30mg (18 pts) in combination with LEN. Median age 62 y (range: 42-82); 76% male; 29% ECOG PS 1; 86% prior atezolizumab/bevacizumab, 24% primary refractory on prior treatment, 70% prior TACE/RFA, 67% extra hepatic disease and 45% had AFP ≥400 ng/mL. Median time on treatment was 5.6 months. Treatment was safe and tolerable with no new unexpected AEs or toxicities. Selected RP2D of fostrox: 30mg. Most common grade ≥3 fostrox related AEs were transient neutropenia and thrombocytopenia (52%), with >70% continuing treatment according to schedule. Fostrox was dose modified in 29%, discontinued in 5% of patients. LEN dose modification was in line with monotherapy use (57% dose reduction). LEN related grade ≥3 AEs were asthenia (10%), hypertension (10%), proteinuria (5%), diarrhea (5%) and hand-foot syndrome (5%). No grade 5 TEAE occurred. Median time to progression (TTP) was 10.9 months (95% CI 4.1-18.1) with a BOR of 24%, duration of response of 7 months and disease control rate (DCR) of 81%.

Conclusion: Fostrox in combination with LEN was tolerable and safe with promising efficacy (TTP = 10.9 months) in a high unmet need HCC population. A randomized phase 2b of fostrox + LEN versus LEN alone is planned to further explore the potential benefit of this combination.

PO2-17-YI
Breaking Barriers for Intensive Care Admission in Patients with Advanced HCC on Immunotherapy

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Background and aims: Intensive Care Unit (ICU) admission is usually denied in patients with advanced hepatocellular carcinoma (HCC) due to the perceived poor prognosis. However, immunotherapy based on immune-checkpoint inhibitors (ICI) has transformed the treatment landscape of these patients. With the increasing use of ICI-based therapies, the role of critical care is becoming more relevant in managing severe adverse events related to this therapy. The outcome of HCC patients requiring critical care requires to be revisited. Our aim is to assess the outcome of patients with advanced HCC treated with IT admitted to ICU.

Method: We evaluated patients treated with ICI combinations across 20 medical centers globally between November 2012 and April 2024. Demographic data, IT types, main causes of admission, organ support, and mortality in the short and medium term were recorded.

Results: Of 1,065 patients, 47 (4.4%) were admitted to the ICU. Most were male (76.6%) with cirrhosis (93.6%), and 59.7% received IT as first-line therapy. The primary reasons for ICU admission were immune-related adverse events (irAE) in 46.8% and variceal bleeding in 29.8%. Median time to ICU admission was 115 days after initiation of IT [38-202], with 51 days [31-137] for irAEs. Half of the patients required organ support, with a median SOFA score of 4 (2-8). ICU mortality was 25.5%. Two thirds of the patients (66%) were alive 28 days post-ICU discharge, with 3- and 6-month survival rates of 83% and 69%. The 61.3% of survivors were rechallenged with IT or started new HCC therapy.

Conclusion: Immune-related adverse events are the main cause of ICU admission in patients with advanced HCC receiving IT. Despite the severity, 66% were discharged, and nearly half resumed the same cancer therapies. These findings highlight the vital role of ICU care in managing HCC patients, challenging the notion of excluding them from intensive care, and emphasizing the need to identify factors that improve prognosis.

PO2-21-YI

The alpha-1 Pi*MZ genotype is an independent risk factor for hepatocellular carcinoma development in patients with advanced chronic liver disease

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Background and aims: Homozygosity for the *SERPINA1 Pi*Z* deficiency allele (i.e., the rare *Pi*ZZ* genotype) is the culprit of alpha-1 antitrypsin deficiency-related liver disease (AATLD), while harbouring a single Z allele (e.g., the common *Pi*MZ* genotype) is the strongest genetic risk factor for ACLD in steatotic liver disease as well as liver transplantation/death in established ACLD. While *Pi*ZZ* genotype predisposes to HCC in the general population, the impact of the *Pi*MZ* genotype on HCC development in patients who have already progressed to ACLD (i.e., those at high risk) has yet to be established.
Thus, we investigated the impact of the *Pi*MZ* genotype on the development of HCC among thoroughly characterized and longitudinally followed ACLD patients.

Method: Patients undergoing hepatic venous pressure gradient (HVPG) measurement and genotyping at the Vienna Hepatic Hemodynamic Lab were included. Patients with HCC at baseline were excluded. Competing risk analyses with HCC as outcome of interest and death/liver transplantation as competing event were performed.

Results: We included 815 patients (mean age 54±11 years; viral hepatitis: 53%, alcohol-related liver disease: 37%, and metabolic-dysfunction associated steatotic liver disease: 10%). Overall, 30 patients (4%) harboured the *Pi*MZ* genotype. During a median follow-up of 47.3 months, 68 patients developed an HCC (8/30 harbouring the *Pi*MZ* genotype).
The *SERPINA1 Pi*MZ* genotype was associated with increased risks of HCC development in univariable as well as in multivariable analysis, the latter accounting for other important factors (i.e., age, sex, removal of the primary aetiological factor, AST, serum albumin level, and HVPG) identified by our analysis (adjusted subdistribution hazard ratio [aSHR]: 3.31 [95%CI: 1.26-8.65]; p=0.015). Other common small nucleotide polymorphisms were not associated with HCC development during follow-up. The impact of the *Pi*MZ* genotype was further confirmed in a multivariable analysis adjusted for the aMAP score (aSHR: 2.94 [95%CI: 1.45-5.97]; p=0.003).

Conclusion: The *SERPINA1 Pi*MZ* genotype is associated with a more than 3-fold increased risk of HCC development in patients who already progressed to ACLD, independently of other HCC risk factors.

PO3-01
Patterns of Extrahepatic Metastasis and Their Impact on Oncological Outcomes in HCC Patients Treated with Atezolizumab Plus Bevacizumab

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Background and aims: We aim to analyze the pattern of extrahepatic metastases (EM) and their impact on oncological outcomes in patients with unresectable hepatocellular carcinoma (HCC) treated by atezolizumab/bevacizumab (Atezo/Bev).

Method: We retrospectively analyzed consecutive patients with intermediate and advanced-stage HCC treated with Atezo/Bev as first-line systemic treatment in 6 centers. We collected clinical, biological and radiological variables at the beginning of Atezo/Bev. We assessed 1) the presence of EM, 2) the numbers of metastasis (none vs 1 vs ≥2) as well as 3) the number of EM sites defined by the number of organs with metastasis. Cox and logistic regression analyses were performed to evaluate the associations between variables and overall survival (OS), progression free survival (PFS) and radiological progression (RECIST v1.1).

Results: 665 patients were enrolled with a median age of 67-year-old and 86% of male; 75% had cirrhosis with HCC classified BCLC A in 1.2%, B in 34% and C in 65% of the cases. Among the whole population of patients, 227 patients (34.1%) had EM. Compared to patients without metastasis, patients with EM were younger with a lower proportion of cirrhosis but had more chronic hepatitis B, prior hepatic surgery and higher CRP and AFP levels. Among the 227 patients with EM, lymph node, lung, peritoneal and bone metastases were observed in 106 (46.7%), 85 (37.4%), 46 (20.3%), 37 (16.3%) cases, respectively. Forty percent of the patients had a unique EM whereas 60% of the patients had ≥2 EM. Moreover, 28.9% of the patients had more than one EM site. The most frequent co-occurring EM sites were lymph nodes/lung (6.5%), lymph nodes/peritoneum (6.0%) and lymph nodes/bone (2.0%). In multivariate analysis, EM were associated with OS (aHR:1.29, 95%CI:1.05-1.59, p=0.02), PFS (aHR:1.37, 95%CI:1.14-1.64, p<0.01) and radiological progression (aOR:1.72, 95%CI:1.19-2.49, p<0.01). In contrast, EM did not significantly affect the proportion of response under Atezo/Bev (p=0.65). No difference in term of oncological outcomes were observed among the different types of EM (lung, bone, lymph nodes,peritoneum) that were all associated with poor oncological outcomes. Metastatic burden (no EM versus one EM versus ≥2 EM) was not significantly associated with OS (p=0.07) but was associated with shorter PFS (p=0.01). Patients with multiple EM sites had shorter OS and PFS (p<0.01).

Conclusion: The presence of extrahepatic metastasis negatively affects the prognosis of patients with hepatocellular carcinoma under Atezo/Bev with shorter overall survival in patients with a high metastatic burden.

PO3-07-YI

Short-period-related lifestyle changes significantly and long-lastingly impact HCC in MASLD via body composition modifications: a multicenter clinical study

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Background and aims: The pandemic lockdown(SARS-CoV2) represented an unrepeatable event forcing the entire population to suddenly change their lifestyle, favoring a drastic reduction in physical activity and an improper diet, with consequences on metabolic homeostasis never experienced before. We investigated the impact of short-period-related lifestyle changes on hepatocellular carcinoma(HCC) occurrence in a Metabolic dysfunction-associated steatotic liver disease(MASLD) context.

Method: A cohort of 222 MASLD patients was followed two years before, during, and after the pandemic. At four time points [January 2018: T0; January 2020: T1; January 2022: T2; January 2024: T3], HCC occurrence, simultaneously with biochemical, nutritional, bioelectrical impedance analysis(BIA), and non-invasive tools(NITs) data, were collected.

Results: During the confinement, a higher overall HCC and Milan-out criteria HCC occurrence emerged [HR:2.521, p:0.01, and HR:13.78, p:0.0009]. At T2, an increase in fats and carbohydrate intake in contrast with reduced weekly physical activity was revealed, associated with an increase in body mass index(BMI), low-density lipoprotein, triglycerides, and insulin levels(all $p < 0.0001$). At T2, an impairment of both Liver Stiffness and Controlled Attenuation Parameter(CAP) emerged(both $p < 0.0001$). Compared with T0 and T1, the BIA evidenced at T2 an increase in Fat Mass (FM) and a reduction of Free Fat Mass(FFM) and Skeletal Muscle Mass(SMM).
The multivariable analysis revealed, contrariwise to the biochemical parameter and NITs variations, the body composition modifications as the variables significantly(all $p < 0.0001$) associated with HCC occurrence.
ROC analysis identified respectively -0.92 Kg (AUC:0.86) and -1.90 Kg (AUC:0.90) for FFM and +1.35 Kg(AUC:0.87) and +1.51 Kg(AUC:0.94) for FM as the best cut-off(BCO) variations(pre-pandemic vs pandemic) in the prediction of HCC overall and HCC Milan-out staged occurrence during the confinement. Consistently, time-to-event analysis revealed a higher incidence of HCC overall occurrence during the pandemic period in patients presenting the body composition modification “combined pattern”(BMCP)[< BCO FFM decrease + > BCO FM increase] compared with individuals showing only one of two variations(p:0.025).
At T3, compared to the pandemic period, simultaneously with improvement in physical activity as well as a decrease in fats and carbohydrate intake, the BMI, insulin levels, CAP, and FM were significantly decreased($p < 0.0001$). Relevantly, in BMCP patients, a higher incidence of HCC overall occurrence(p:0.048) continued to emerge.

Conclusion: The confinement-related lifestyle changes impacted MASLD evolution via body composition modifications, negatively influencing the overall and HCC Milan-out criteria occurrence even after the restoration of pre-confinement conditions.

PO3-08-YI
SHiNE-UK a national evaluation of hepatocellular carcinoma surveillance and management

On behalf of The Trainee Collaborative for Research and Audit in Hepatology UK (ToRcH-UK)[1]
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Background and aims: Hepatocellular carcinoma (HCC) is a rapidly growing cause of cancer mortality despite advancing treatment strategies, with the mounting burden of chronic liver disease a major concern. Earlier diagnosis and improved access to curative treatments are a key, with concerted efforts towards 'precision surveillance' via risk-prediction modelling and better diagnostics. However, low adherence to surveillance standards is widely reported, with measurement fraught with heterogeneity and limitations, contributing to the debate around effectiveness. This study aims to evaluate the performance of HCC pathways, to guide service improvement and pave the way for future innovations.

Method: A multi-centre retrospective cohort study was conducted. Eligible cases were identified and local audit performed with follow up 1/1/22 - 30/1/24. REDCap facilitated data collection and query resolution. Analysis was performed in RStudio. Surveillance adherence was measured using a modified proportion of time covered (mPTC) method, assuming 6-months cover per test. Regression analysis was performed to identify factors associated with adherence and Barcelona Clinic Liver Cancer (BCLC) stage.

Results: A total of 68 hospitals participated across four nations with all levels of hepatology service represented. Surveillance pathways included a dedicated imaging procedure code (26%), pathway navigators (21%), interventional programmes (15%) and automated recall (9%). LI-RADS was used for ultrasound (11%) and CT/MRI (64%). Patients in surveillance (n = 1,674) had a median mPTC for alpha-fetoprotein (27.5%), surveillance scans (68.4%) and all abdominal imaging (72.9%). Over two years, HCC incidence in surveillance was 2.5%, with 41% at early stage (BCLC 0-A). Cumulative incidence by aMAP risk—low (0%), medium (1.1%), high (5.2%)—was significant ($p < 0.001$), with no events in the low-risk group. For incident HCC (n = 723), routes to diagnosis included cirrhosis (48%) and surveillance (42%), with presentation via incidental (38%), surveillance (37%) and symptomatic (24%) channels. Rates of early-stage diagnosis (BCLC 0-A) and curative first-line treatment were 44% and 27%, respectively. First-line treatment utilisation was 79%, with reasons for non-utilisation identified. Absence of surveillance was significantly associated with late-stage diagnosis (BCLC B-D; $p = 0.025$).

Conclusion: This national evaluation has determined the current performance of HCC surveillance and treatment pathways in the UK. We propose mPTC should be the gold-standard for measuring surveillance adherence given its precision. Access to curative treatment is low but surveillance in its current form is associated with better outcomes. Future efforts should harness both service improvement and emerging innovations to improve equitable access to curative treatment.

PO3-19

Sorafenib combined with transarterial chemoembolization compared with sorafenib alone in advanced hepatocellular carcinoma (SELECT): a multicenter, phase 3, randomized, controlled trial

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Background and aims: The prognosis of patients with advanced-stage hepatocellular carcinoma (HCC) is extremely poor. This trial was designed to evaluate the efficacy and safety of sorafenib combined with transarterial chemoembolization (TACE) compared with sorafenib alone for advanced-stage hepatocellular carcinoma.

Method: This phase 3, SELECT randomized, controlled trial was conducted at twelve centres in China (NCT01906216). Eligible patients were advanced-stage hepatocellular carcinoma (Barcelona Clinic Liver Cancer stage C), Child-Pugh class A liver disease, Eastern Cooperative Oncology Group performance statuses of 0 or 1. The primary endpoint was overall survival, and analysis was by intention-to-treat and per-protocol. Secondary endpoints included time to progression, tumor response rates and safety. Overall survival, and time to progression were analyzed with Kaplan-Meier analysis, hazard ratios (HRs) and 95% confidence intervals (CIs). We used R version 4.4.1 for statistical analyses.

Results: Between September 7, 2013 and December 4, 2019, 199 patients were randomly assigned, 99 to the combination group and 100 to the sorafenib alone group. Protocol adherence was 86% (85 of 99 patients) in the combination group and 56% (56 of 100 patients) in the sorafenib alone group. At a median follow-up of 13.6 months (IQR 6.8-28.2), there was no significant difference in overall survival in the intention-to-treat population (14.9 months [95%CI 10.5-19.3] in the combination group versus 11.9 months [95%CI 9.0-14.8] in the sorafenib alone group; HR 0.862 [95%CI 0.645-1.150]; p=0.312). However, in per-protocol population, median overall survival was 14.6 months (95%CI 11.3-17.9 months) in the combination group, compared with 7.4 months (95%CI 4.3-10.5 months) in the sorafenib alone group (HR 0.539 [95%CI 0.378-0.769]; p=0.001). The endpoints of time to progression and objective tumor response rates were all met in the intention-to-treat and per-protocol population. The overall incidence of adverse events was similar between these two groups.

Conclusion: Our findings indicate that sorafenib combined with transarterial chemoembolization is an effective intervention for advanced-stage hepatocellular carcinoma. The significance of overall survival of systemic drug combined with locoregional therapy for advanced-stage HCC management deserve further evaluation.

PO3-20
Machine and deep learning for non-invasive detection of esophageal varices in hepatocellular carcinoma patients

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Background and aims: Portal hypertension (PH) screening in advanced hepatocellular carcinoma (HCC) remains challenging. As current Baveno criteria lack sensitivity, endoscopy is required to detect esophageal varices (EV) to determine need for variceal bleeding prophylaxis. We aim to develop and validate a non-invasive classifier for EV status in HCC patients to avoid systematic endoscopy, utilizing deep learning (DL-imaging model) on contrast-enhanced CT scans (CECT) alongside clinical-biological data and imaging findings at baseline (baseline model).

Method: Data from 223 HCC patients eligible for Atezolizumab-Bevacizumab (AtezoBev) from three French hospitals with arterial phase CECT and no prior liver transplant were collected. Endoscopy for EV assessment was conducted within six months before starting AtezoBev. Patients were divided into training (AVC, PSL) and validation (SAT) cohorts. Abdominal axial CT features were extracted for DL-imaging model training and validation for EV classification. For the baseline model, a logistic regression model with LASSO-selected signature was trained and validated. Model performance was assessed by area under the receiver operator curve (AUROC).

Results: Median patient age was 65.6 years (84% male). Cirrhosis was present in 83.8% of patients, 49% had viral infection, alcohol consumption was present in 46% and metabolic syndrome in 37% (74% had mixed cause of liver disease). 74% were Child-Pugh A (MELD 8, 14% ALBI score 3). Median platelet count was 164,000/mm^3, total bilirubin was 14.0 uM/dL, INR was 1.14, albumin was 35.0 g/dL and creatinine was 72.0 uM. 53% had EV and 21% had history of ascites. Collaterals at imaging were present in 55%. HCC was multinodular in 77% of cases, with median size of 60 mm for largest lesion, 38% had infiltrating HCC and 41% vascular invasion, 60.5% were BCLC-C and 50% were treatment-naive for HCC. The DL-imaging model was able to classify EV status with AUROCs (median [95% confidence interval]) of 0.76 [0.57-0.91] in validation. For the baseline model, the signature was composed of 6 features: platelet count with log-odds ratio (LOR) of -0.22, total bilirubin (LOR: 0.40), presence of collaterals (LOR: 2.74), albumin (LOR: -0.19) and creatinine levels (LOR: -0.29) and INR (log-odds ratio: 0.09), showing an AUROC of 0.95 [0.84-1.00] in validation.

Conclusion: The baseline model classified EV status with very good performance, outperforming the DL-imaging model. High importance for collateral vessel presence on CECT-scan was observed in the baseline model, suggesting that patients with collaterals may benefit from AVB prophylaxis with beta-blockers, potentially eliminating the need for endoscopy.

PO3-21-YI

The “A-B-C” cluster-based classification reveals distinct outcome trajectories in patients with hepatocellular carcinoma under atezolizumab/bevacizumab

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Background and aims: Patients with unresectable hepatocellular carcinoma (HCC) treated with atezolizumab and bevacizumab exhibit variable outcomes. We aimed to identify clinically meaningful subgroups using machine learning to assess their association with oncological outcomes

Method: In this international multicenter study across 12 centers, we included patients with unresectable HCC receiving first-line atezolizumab and bevacizumab. Baseline clinical, biological, and tumor characteristics were collected. Hierarchical clustering analysis was performed on a derivation cohort and validated in a validation cohort. Clusters were correlated with overall survival (OS), progression-free survival (PFS) and radiological response per RECIST 1.1

Results: Among 1,399 patients (85% male; 75% with cirrhosis; 39% with portal vein invasion; 35% with metastasis), divided into derivation (n = 958) and validation (n = 409) cohorts, we identified three distinct subgroups: Cluster A (47.5% of patients): older age, higher body mass index, preserved liver function, multiple small tumors; Cluster B (11%): presence of VP1/VP2 portal vein thrombosis, higher hepatitis B and lower metabolic syndrome prevalence; Cluster C (41.2%): moderate liver dysfunction, elevated alpha-fetoprotein levels, and either VP3/VP4 portal vein thrombosis or high tumor burden. Cluster A had longer OS (median not reached) compared to Clusters B (18.2 months) and C (14.1 months; $p<0.0001$), lower rates of progressive disease (25.1% vs. 34.3% and 41.2%; $p<0.0001$), and longer PFS (median 10.93 vs. 7.73 and 6.10 months; $p<0.0001$). Clusters correlated with prognosis across BCLC stages and specific patterns of disease progression ($p<0.001$).

Conclusion: The Atezolizumab-Bevacizumab-Cluster (A-B-C) classification identified subgroups of patients with different prognostic trajectories. These clusters were associated with survival outcomes and patterns of disease progression, potentially informing personalized therapeutic strategies and clinical trial design in patients with unresectable HCC.

PO3-22-YI

The prognostic impact of comorbidities in patients with hepatocellular carcinoma: a multicenter observational study

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Background and aims: Charlson Comorbidity Index (CCI) could be used to assess the burden of comorbidities in different clinical settings, but few data are available in patients with hepatocellular carcinoma (HCC). We aimed to evaluate the CCI as a prognostic predictor in HCC patients, as well as to derive a modified CCI specifically developed in this patient population.

Method: From the ITA.LI.CA database, data of 8945 patients diagnosed with HCC after January 2000 were retrieved. The association between CCI and the probability of receiving a curative treatment and the prognostic impact of CCI were evaluated through multivariate logistic regression and Cox regression analyses, respectively. In order to recalibrate the CCI, the independent effect on survival of each comorbidity of the original CCI was assessed with a multivariate Cox regression analysis. The CCI-HCC score was calculated adding the points for each independent comorbidity (equal to the hazard ratio rounded to the nearest integer) to the points derived from age (as in the original CCI).

Results: The median value of CCI in the entire cohort was 7 (6 – 9). Higher CCI values were independently associated with a lower probability of receiving a curative treatment (OR 0.88, 95% CI 0.85 – 0.90) and with a worse overall survival (HR 1.05, 95% CI 1.03 – 1.07). When individual components of CCI were analyzed, myocardial infarction (HR 1.15, 95% CI 1.06 – 1.25), chronic kidney disease (HR 1.17, 95% CI 1.04 – 1.32) and chronic obstructive pulmonary disease (HR 1.18, 95% CI 1.08 – 1.28) emerged as independent predictors of survival, and were included as variables in the CCI-HCC score. The median overall survival was 56.0 months (95% CI 48.6 – 63.4), 46.0 months (95% CI 43.9 – 48.1) and 31.5 months (95% CI 29.2 – 33.9) in patients with CCI-HCC score of 0 – 1, 2 – 3 and ≥ 4, respectively ($p<0.001$). Moreover, the CCI-HCC score maintained an independent association with prognosis (HR 1.03, 95% CI 1.01 – 1.06). In a competing risk analysis, the CCI-HCC score was independently associated with death from not liver-related causes (HR 1.16, 95% CI 1.09 – 1.23), but not from HCC progression (HR 0.96, 95% CI 0.92 – 1.01) or liver dysfunction (HR 1.05, 95% CI 0.99 – 1.12).

Conclusion: In patients with HCC, the burden of comorbidities as assessed with the CCI is associated with a diminished survival. We derived in this specific population a modified simple comorbidity index (CCI-HCC) which may be useful to assess prognosis of HCC patients.

PO3-23-YI
The changing epidemiology of patients with HCC receiving a first-line systemic therapy: insights from ARPES and ARTE databases (2008-2024)

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Background and aims: The epidemiological characteristics of patients starting first-line systemic therapy for hepatocellular carcinoma (HCC) have changed over time. This study examines shifts in demographics and disease profiles since the first systemic therapy was introduced.

Method: We compared baseline characteristics of patients receiving frontline systemic treatments from two Italian multicenter nationwide datasets of systemic treatments: ARPES (sorafenib, 656 patients, 2008-2019) and ARTE (atezolizumab and bevacizumab [atezo/bev], 393 patients, 2022-2024). ARPES included cardiometabolic risk factors, allowing reclassification from NAFLD to MASLD.

Results: Compared to ARPES, patients in ARTE showed a higher prevalence of females (19.9% vs. 15.1%; p=0.047), a trend toward older age, and an increase in single-etiology MASLD (17.6% vs. 8.8%, p<0.001), with fewer viral cases. More patients with HCV were in sustained virologic response at treatment start (72.8 vs 9.1%, p<0.001). Additionally, patients included in ARTE had better liver function (ALBI grade-1: 52.6 vs 18.7%, p<0.001), highest rate of no prior surgical/locoregional HCC treatments (38.5% vs. 28.1%, p<0.001), less prevalent BCLC-C stage due to fewer cases with macrovascular invasion (31.0% vs. 43.1%, p<0.001) and similar rate of metastatic disease. Lower likelihood of previous surgical/locoregional treatments was confirmed in the intermediate-stage subgroup (19.9 vs 35.2%, p<0.001). Tumors larger>6 cm (14.9% vs. 10.0%, p<0.001) and ECOG-PS>0, (32.0% vs. 23.3%, p<0.001) were also more common in the ARTE database.

Conclusion: The increased prevalence of MASLD, a decline in viral cases, high SVR rates in HCV, and less previous locoregional treatments are likely to contribute to better liver function and more patients with intermediate stage. The challenges of surveillance in patients with MASLD may explain the increase in cases with no prior treatment, larger tumors, and higher ECOG-PS scores.

PO4-01-YI

Prediction of nodal status on preoperative MRI for patients with intrahepatic cholangiocarcinoma: an apparent diffusion coefficient-based machine learning model

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Background and aims: Lymph node (N) status is a critical predictor of survival in patients with intrahepatic cholangiocarcinoma (iCCA). However, when no positive lymph nodes are detectable at preoperative imaging, the benefit of lymphadenectomy remains debated, given the risk of postoperative complications. This study aims to develop a machine-learning model to preoperatively predict N status, potentially guiding surgical strategy.

Method: MRI data from a monocentric cohort of iCCA surgical patients between 2011 and 2023 were retrospectively analyzed. Two radiologists, blinded to surgical and pathological details, independently assessed lymph nodes ≥ 5mm in hepatic locoregional nodal stations. For each identified lymph node, measurements of diameter and corresponding Apparent Diffusion Coefficient (ADC) values were extracted from MRI scans obtained within two months before surgery. Inter-rater agreement was confirmed via Bland-Altman plots, allowing the calculation of ADC and diameter mean values among the two sets of observations. A Support Vector Machine (SVM) model was trained to classify N status using MRI and clinical data, with class weights to address class imbalance and a radial kernel for optimized performance. Model tuning was performed with leave-one-out cross-validation. The primary endpoint was the model area under the curve (AUC), evaluated by ROC analysis.

Results: A total of 43 patients met the study criteria. From them, 47 lymph nodes were matched to pathological reports, of which 13 (27.7%) were metastatic. ADC values significantly differed between N0 and N1 groups (1177 vs 1001 mm2/s, p = 0.029), while diameter did not (14.2 vs 20.0mm, p= 0.178). Multifocal disease was the only clinical variable selected by multivariate logistic regression (OR 8.46, p = 0.06). The SVM model, incorporating lymph node diameter, ADC values, and multifocal disease status, achieved an AUC of 0.91 (95%CI: 0.83-0.99) and an accuracy of 85.7% (95%CI: 71.5-94.6). Model performance metrics included a kappa value of 0.692, sensitivity of 82.8%, specificity of 92.3%, positive predictive value of 96.0%, and negative predictive value of 70.6%. The model's predictive performance was significantly superior to the No Information Rate of 69.1% (p = 0.011).

Conclusion: Lymph node diameter alone does not reliably discriminate pathological status. Integrating diameter with ADC values, the proposed machine-learning model effectively predicts N status in the preoperative setting. This approach can support treatment planning, allowing lymphadenectomy to be reserved for patients most likely to benefit from the procedure.

PO4-03-YI

Efficacy and safety of atezoliumab/bevacizumab for hepatocellular carcinoma in a real-world prospective cohort: a 2024 update

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Background and aims: Atezolizumab/bevacizumab (atezo/bev) is a standard-of-care for patients with unresectable hepatocellular carcinoma. Most efficacy and safety data, however, stem from clinical trials, with fewer evidence from real-world practice. We aimed to provide real-world clinical data of patients with HCC treated with atezo/bev.

Method: We analyzed clinical data and outcomes for patients with enrolled in the ARTE database, a nationwide, multicenter Italian dataset focused on immunotherapy treatments for HCC, covering the period from March 2022 to November 2024. The ARTE study group prospectively collects data from patients who began atezo/bev therapy outside the context of clinical trials.

Results: Data from 397 patients across 15 centers were included. The majority had advanced HCC (59.9%). Sixty patients (15.1%) presented with one or more conditions outside the IMbrave150 enrollment criteria (thrombocytopenia <70,000/mmc [n=26], concurrent/recent malignancy [n=13], concurrent anticoagulation [n=9], arrhythmia [n=8], HIV infection [n=4], chronic heart failure [n=2]). Hepatitis C virus (HCV) was the most common etiology (45.1%), followed by metabolic-associated steatotic liver disease (MASLD, 33.0%), alcohol-related liver disease (ALD, 27.7%), and hepatitis B virus (HBV, 15.9%). 103 patients (25.9%) had multiple risk factors. Performance status (ECOG-PS) >0, macrovascular invasion (MVI), extrahepatic spread, and alpha-fetoprotein (AFP) >400 ng/ml were observed in 31.5%, 32.7%, 39.0%, and 28.0% of patients, respectively. Fifty-three patients (13.4%) received non-systemic therapies after starting atezo/bev, including surgical (transplant n=8, resection

n=4), percutaneous (n=9), and trans-arterial procedures (n=18), or non-liver-directed radiotherapy (n=20).
Median overall and progression-free survivals were 20.4 (95% CI 17.8-23.0) and 9.6 months (8.4-12.8), respectively. ECOG-PS >0, MVI, AFP >400 ng/ml, ALBI grade >1, and neutrophils-to-lymphocytes ratio >3 were independent negative prognostic factors.
The most common treatment-related adverse events (AEs) included fatigue (45.1%), hypertension (24.7%), anorexia (17.1%), and diarrhea (13.9%). Most common treatment-related Grade 3-4 AEs were: hypertension (5.3%), variceal bleeding (3.5%), increased aminotransferases (2.8%), and digestive non-variceal bleeding (2.5%)

Conclusion: Real-world findings confirm previous efficacy and safety data for atezo/bev. Multiple HCC risk factors, comorbidities, and combination with surgical/locoregional treatments are common in clinical practice and warrant dedicated studies.

PO4-07
A Dynamic Contrast Enhanced Ultrasound Based Risk Prediction Model for the diagnosis of Hepatocellular Carcinoma in the grey area of CEUS-LI RADS: The PERSoN4 Model

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Background and aims: Hepatocellular carcinoma (HCC) in patients with liver cirrhosis is characterized by a distinct dynamic vascular pattern, marked by arterial hyperenhancement followed by late and mild wash-out in the portal-venous phase. However, a substantial proportion of HCC cases exhibit atypical imaging features. Dynamic Contrast-Enhanced Ultrasound (D-CEUS) employing standardized software emerges as a promising tool, potentially enhancing the accuracy of tumor perfusion assessment. This study aims to investigate the utility of integrating D-CEUS into the diagnostic algorithm for HCC in patients currently identified as candidates for liver biopsy.

Methods: From January 2021 to November 2023, consecutive patients with chronic liver disease and liver nodules candidated to liver biopsy were enrolled in this prospective monocentric study. A validation cohort composed of cirrhotic patients from another hospital was identified. Contrast enhanced ultrasound (CEUS) was perfomed in all patients before biopsy and assessed by CEUS LI-RADS. Clips were examined by VueBox® software to obtain the time intensity curves. Baseline clinical characteristics and D-CEUS based quantitative parameters were compared among the different histological entities. Univariable analysis was employed, and relevant parameters were incorporated into a logistic regression model for HCC diagnosis. The diagnostic accuracy of the identified model was evaluated by Receiver Operating Characteristic (ROC) curve and relative Area Under the Curve (AUC).

Results: A total of 58 patients (mean age 67, 36 men) were enrolled, including 32 HCC, 15 intrahepatic cholangiocarcinoma (ICC) and 11 liver metastasis (LM). According to CEUS LI-RADS, 45 patients were classified as LI-RADS M, 5 as LI-RADS 3, and 8 as LI-RADS 4. Statistically significant differences between HCC and non-HCC patients were observed for variables such as the number of nodules ≥4 (p=0.03), irregular margins (p=0.01), peripheral rim-like hyperenhancement (p=0.002), and Peak Enhancement (PE) percentage change (p=0.006). The optimal logistic regression model was identified incorporating the following predictive variables: sex, number of nodules ≥4, peripheral rim-like hyperenhancement, and PE percent change. The model showed high accuracy (AUC 0.91) for the diagnosis of HCC. In the validation cohort the model confirmed high accuracy (AUC 0.73).

Conclusion: A risk assessment model, combining clinical and D-CEUS data, has the potential to enhance the diagnostic performance of standard CEUS LI-RADS criteria for non-invasive HCC diagnosis in high-risk patients. If our data will be confirmed in larger multicenter studies, the proposed diagnostic model could revolutionize the non-invasive diagnosis of HCC in over 60% of patients currently considered candidates for liver biopsy.

PO4-10
Characterization of hepatic lesions by LI-RADS during the surveillance of advanced liver disease in the prospective cohort of cases with congenital univentricular heart after Fontan palliation

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Background and aims: The congenital univentricular heart (UH) is a rare disease corrected with the palliative Fontan's intervention, that restores the survival from childhood up to adult life. Unluckily, these patients develop a series of complications, among all, the Fontan associated liver disease (FALD), that causes the occurrence of liver cirrhosis and hepatocellular carcinoma (HCC), and negatively impacts the life expectancy. Our primary goal was to identify the presence of severe FALD and cardiogenic cirrhosis in UH patients by VCTE stiffness measurement of liver (LSM) and spleen (SSM), and the liver fibrosis scores (FORNS, liver LSPS and spleen SSPS platelet score and the novel Padua-Cardiogenic Cirrhosis or P-CC index). Secondarily, to characterize all the liver lesions proposing the LI-RADS CT/MRI system (LRS), particularly of those with arterial phase hyperechoic enhancement (APHE).

Method: 169 patients (102M/67F, aged 27,2±12.0 yrs) born with UH corrected with Fontan circuit were prospectively followed-up from 2019 (FU 26.8±17.6 mos). Patients underwent: a) specific lab-test profile, b) LSM and SSM by Fibroscan (Echosens, Paris) and c) liver imaging examination by US, CT and MR. ROC curves were built to select the most accurate scores cut-off to diagnose and grade FALD.

Results: All the scores used (FORNS with cut-off ≥4, LSPS ≥1.4, SSPS ≥2.3, LS ≥20.2 and SS ≥30.7) showed high sensitivity and specificity, improving diagnostic accuracy of FALD staging (all, $p<0.01$). By identification of selective criteria related to advanced liver disease and portal hypertension, we applied a novel P-CC index, that predicted a low/moderate-risk profile (HR 0.30; CI 0.18-0.52) in 83 patients and a high-risk profile (HR 3.29, CI 1.93-5.60) in 86 (51%). During FU, 12 patients (7.1%) died, 14 received OHT (8.2%) and 5 had occurent HCC (2,9%). Lastly, based on the characterization of liver lesions found in 57 cases (36%), we grouped by LRS: 26 cases with LR-1 or 2 (benign focus), 23 with LR-3 (suspect) and 8 with LR-4 or 5 (malign). Cases with APHE, grouped by LR-3-4-5 classes (31 cases, 54%) appeared significantly associated with a more advanced FALD (80% vs. 46%; $p=0.01$) and longer time since Fontan (29.3±9.4 vs. 20.8±8.8; $p=0.02$).

Conclusion: The novel P-CC index may help to predict the development of HCC in high risk patients. The active surveillance by imaging to characterize the APHE liver lesions with a malignant course, appears reliable when applying LRS, even for lesions that occur in a vascular-congestive setting.

PO4-12
Data from a multinational registry on liver transplantation after immune checkpoint inhibition in hepatocellular carcinoma patients

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Background and aims: Immune checkpoint inhibitor (ICI) combination therapies have become standard of care in advanced stage hepatocellular carcinoma (HCC) and in selected patients with intermediate stage HCC. Liver transplantation (LTx) without standard exception criteria represents a possible therapeutic option in selected BCLC B patients who respond well to ICI treatment. However, the risk of potentially fatal allograft rejection following ICI treatment remains a significant concern. To investigate outcomes of LTx after ICI therapy, data from an international multicenter registry were analyzed.

Method: Data were obtained from ten European transplant centers from patients with HCC undergoing LTx after ICI therapy. Collected data included patient and tumor characteristics, details on ICI regimens, and time interval between ICI and LTx. Overall and rejection-free survival rates were analyzed using the log-rank test.

Results: Out of 15 patients who underwent LTx after ICI treatment, 12 received atezolizumab/bevacizumab. At 12 months, survival was 75% (9 out of 12 patients), with a median follow-up of 535 days. The median wash-out time for ICI Inhibitors was 143 days (range 13 to 680 days). There were four documented cases of rejection: one fatal rejection occurring 21 days post-LTx, and three non-fatal rejections, at 8 days, 27 days, and 5 months post LTx, respectively; all three of them were successfully treated with intensified immunosuppression. Notably, the only fatal rejection observed occurred after a short ICI wash-out period of only 13 days. The other rejections had a wash-out time of 74, 136, and 218 days.

Conclusion: In this cohort, a rejection rate of 27% was observed following LTx after ICI therapy. The short interval between ICI and LTx observed in the fatal rejection case suggests that timely discontinuation of ICI treatment prior to LTX appears to be crucial to avoid rejection. In summary, this study demonstrates that LTx can be safely performed following ICI therapy in the majority of patients.

PO4-14
HIPERIA Study: Impact of clinically significant portal hypertension on the prognosis of patients with compensated cirrhosis and hepatocellular carcinoma undergoing systemic treatment

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Background and aims: In our setting, hepatocellular carcinoma (HCC) typically presents in patients with cirrhosis, making prognosis dependent on both the tumor stage and the severity of liver disease. Clinically significant portal hypertension (CSPH) marks a critical milestone in the natural history of compensated cirrhosis, identifying patients at risk of decompensation and demonstrating a known negative impact on survival in those with HCC undergoing surgical treatment. However, its relevance in the prognosis of patients with HCC undergoing systemic treatment has not been established. This study aims to analyze the impact of CSPH on overall survival and the systemic treatment window in patients with compensated cirrhosis and HCC.

Method: Multicenter retrospective study of patients with HCC and compensated cirrhosis receiving systemic therapy. Primary objective: overall survival; secondary objective: systemic treatment window, defined as the duration of treatment until symptomatic progression or death. Symptomatic progression was defined as severe hepatic decompensation and/or ECOG 3-4 that warrant discontinuation of

treatment at the discretion of the treating physician. CSPH was described according to Baveno VII criteria. Mortality and treatment window were analyzed as time-to-event variables with adjusted Hazard Ratios (HRs) through Cox regression for prognostic variables (age, etiology of liver disease, MELD, ALBI, BCLC stage, and type of systemic treatment). Survival was analyzed using Kaplan-Meier curves.

Results: Between January 2015 and January 2023, 663 patients were included, with a median follow-up of 13.6 (6,0-26,0) months (86.6% male, median age 66 years, 49% HCV-positive, 40% alcohol cirrhosis, median MELD score 8, Child-Pugh class A 100%, ALBI grade 1 in 39%, ALBI grade 2 in 60%, BCLC stage B in 39%, BCLC stage C in 56%). Systemic treatments included sorafenib (86.9%), atezolizumab-bevacizumab (7.6%), lenvatinib (3.9%), and nivolumab (1.7%). 62% had CSPH, with esophageal varices present in 280 patients (42.2%). The presence of CSPH was independently associated with decreased survival (median OS 13.0 [11.1-14.7] vs 21.7 [18.8-25.9] months), adjusted HR (aHR): 1.37 (1.07-1.77), p = 0.014, and a shorter treatment window (10.21 [8.70-11.76] vs 18.82 [15.89-21.02] months), aHR 1.37 (1.05-1.79), p = 0.02.

Conclusion: The presence of clinically significant portal hypertension (CSPH) stratifies the prognosis of patients with compensated cirrhosis and HCC undergoing systemic treatment. CSPH identifies a patient population with poorer survival and a shorter therapeutic opportunity window.

PO4-18
Annotation-free classification of liver cancer in whole slide images with multi-view feature representation-based one-way domain adaptation

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Background and aims: Deep learning (DL)-based digital pathology requires annotating tumor regions in whole slide images (WSIs) through manual examination by pathologists, and a lot of annotated WSIs are necessary for training DL models. While public datasets can meet these needs, in-house datasets are underutilized due to their limited annotations. A potential solution is to apply DL models trained on public datasets to in-house datasets, but the heterogeneity between datasets can hinder effectiveness. This problem can be alleviated by domain adaptation, where different datasets are transformed to be seen as a single set by matching data properties, such as distribution and manifold. Nevertheless, existing methods need improvements in two aspects. First, they need to minimize information loss from the public datasets by only transforming in-house datasets, preserving the integrity of public datasets. Second, they should be specialized in the unique characteristics of WSIs, particularly spatial information and rotational consistency, where the former allows a better representation of tissue images, while the latter ensures robustness across different orientations of the same tissue. Accordingly, we propose a novel framework for annotation-free classification of tumors in WSIs with multi-view feature representation-based one-way domain adaptation.

Method: The proposed method consists of three phases: multi-view feature representation, one-way domain adaptation, and tumor patch classification. First, we extract spatially informed and rotationally consistent features of patches from the original patches of WSIs in public and in-house datasets. Next, our method finds the feature projection matrix, in which the extracted features of the in-house dataset are transformed to be represented similarly to those of the public dataset. Finally, the original patches in the in-house WSIs are transformed by the projection matrix, and then, the transformed patches are applied to the tumor classifier that is pre-trained on the public WSI dataset.

Results: Our method was applied to two datasets of the liver tissue WSIs, which were collected from the Pathology AI Platform (PAIP) with 393 slides and the Yonsei University Health System (YUHS) with 102 slides. The experimental results demonstrated that the proposed method outperforms ten existing domain adaptation methods by 24% and 13% for domain adaptation and tumor classification, respectively.

Conclusion: In this study, we developed a novel machine learning-based framework for annotation-free classification of tumors in WSIs. Our findings suggest that the developed method can contribute to the clinical field as a diagnostic aid for more efficient and precise screening of patients with liver cancer.

PO4-21
Prognostic Value of Imaging-Detected Immune-Related Adverse Events in Unresectable Hepatocellular Carcinoma Treated with Atezolizumab and Bevacizumab

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Background and aims: Following the success of the IMbrave150 trial, the combination of atezolizumab and bevacizumab has been established as the standard first-line treatment for patients with unresectable hepatocellular carcinoma (HCC), owing to its significant improvement in overall survival. Immune-related adverse events (irAEs) during treatment with this regimen have been linked to better prognosis. However, the imaging characteristics of irAEs and their potential prognostic implications during atezolizumab plus bevacizumab therapy remain underexplored. This study aimed to evaluate the imaging findings related to irAEs and their impact on prognosis.

Method: In this retrospective cohort study, consecutive patients who received atezolizumab in combination with bevacizumab as a first-line treatment for unresectable HCC were included. All available imaging studies performed during the course of treatment were reviewed to identify irAEs. The primary objectives were to characterize the imaging findings indicative of irAEs and to assess the correlation between these findings, tumor response, and overall survival. Statistical analysis was performed to compare disease control rates and survival outcomes between patients with and without irAEs.
Results: A total of 198 patients (168 males and 28 females, median age 62.5 years) treated between November 2020 and June 2023 were included in the analysis. Of these, 27 patients (13.6%) exhibited irAEs on imaging, including pulmonary infiltration (n=15), enterocolitis (n=7), mesenteric panniculitis (n=3), and lymphadenitis (n=2). The presence of irAEs identified on imaging was significantly associated with a higher disease control rate (100% in patients with irAEs vs. 67.3% in those without irAEs; P<0.001). Additionally, patients with imaging-detected irAEs demonstrated markedly improved overall survival rates, with estimated 1- and 2-year survival rates of 84.9% and 51.6%, respectively, compared to 50.0% and 22.8% in patients without irAEs (P<0.001).

Conclusion: The development of irAEs identified through imaging during atezolizumab plus bevacizumab treatment in patients with unresectable HCC was significantly associated with improved disease control and overall survival.

PO4-23
Proton-pump inhibitors and Gut Microbiome are Associated with Survival in Hepatocellular Carcinoma under Immunotherapy

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Background and aims: The efficacy of immunotherapy may be influenced by the gut microbiome. Primary aim was to study the role of proton-pump inhibitors, antibiotics and gut microbiome composition on survival in patients with hepatocellular carcinoma under immunotherapy.

Method: This is an exploratory analysis of a multicenter phase I/IIa trial of patients with hepatocellular carcinoma treated with the regorafenib and nivolumab in second line. Fecal samples were collected before starting nivolumab and profiled by 16S ribosomal RNA sequencing. Main outcome was overall survival.

Results: Median age of the 40 patients analyzed was 65.4 years, 82.5% male, 60% with cirrhosis, all ECOG-PS-0 and 62.5% in BCLC-C stage. Twenty received proton-pump inhibitors before immunotherapy and 12 antibiotics within the 12 months prior to treatment start. Cox regression analysis showed that, BCLCpC2 (HR 3.0, 95%CI 1.1-8.3), AFP> 400 (HR 2.7, 95%CI 1.1-6.5), obesity (HR 3.1, 95%CI 1.2-7.8) and proton-pump inhibitors (HR 3.0, 95%CI 1.1-8.3) were associated with higher risk of death. Use of antibiotics within 12 months lowered mortality risk (HR 0.3 95%CI 0.1 -0.99). After IPTW analysis and adjustment for confounders, proton-pump-inhibitors use (HR 4.4, 95%CI 1.6-12.1) was associated with higher risk of death. Multivariate analysis including microbiome profile showed that higher abundance of Streptococcaceae, Gemellaceae, Sutterellaceae and Synergistaceae families was associated with higher risk of death. Proton-pump-inhibitors use was associated with higher abundance of Streptococcaceae and lower abundance of Lachnospiraceae.

Conclusion: Use of proton-pump inhibitors and gut microbiome composition are associated with survival in hepatocellular carcinoma patients under immunotherapy. The use of this class of drug should be avoided in the absence of a mandatory indication

PO5-01
Diagnosis of patients with fibrolamellar carcinoma: a Dutch nationwide study

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Background and aims: Fibrolamellar carcinoma (FLC) is a rare primary liver cancer characterized by abundant eosinophilic cytoplasm and lamellar fibrotic bands. Adequate diagnosis is important for prognosis and treatment. The current study describes the diagnosis of fibrolamellar carcinoma in a Dutch historical cohort.

Method: Adult patients diagnosed with FLC between 1990 and 2020, with pathology slides and clinical data available, were included through the Netherlands Cancer Registry and Automated National Pathological Anatomy Archive. Two expert hepatopathologists revised histopathology and immunohistochemistry (CD68 and CK7).

Results: In total, 52 adult patients, 25 (48%) male, diagnosed with FLC were included. Biopsies were available for 30 patients (58%) and resection specimens in 22 patients (42%). Upon expert review, in nine patients (17%) diagnosis FLC was unequivocally confirmed. Patients diagnosed with unequivocal FLC had a mean age of 26 years. Four additional lesions harbored characteristics of both FLC and conventional hepatocellular carcinoma (HCC). Three patients exhibited morphological features suggestive of FLC, yet with negative CD68 staining. In the remaining 36 patients diagnosis was revised in cholangiocarcinoma (CCA, n=6, 12%), conventional HCC (n=25, 48%) or HCC/CCA (n=5). The lesions identified as conventional HCC were of steatohepatitic (n=11), scirrhous (n=9), and chromophobe (n=5) subtypes

Conclusion: The presence of fibrotic bands in steatohepatitic and scirrhous HCC can lead to misdiagnosis of FLC as conventional HCC. This could have important treatment consequences as there is a tendency towards surgical treatment of FLC if feasible. Contrarily, evidence supporting the efficacy of systemic treatments for FLC remains limited. All in all, our Dutch historical cohort underlines the challenging diagnosis of FLC and emphasizes the critical role of expert review in accurate diagnosis.

PO5-02

ALBI grade and serum PIVKA-II changes during treatment drive clinical outcomes among atezolizumab-bevacizumab (ATZ/BEV) treated HCC patients with stable (SD) or progressive (PD) disease

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Background and aims: The majority (> 65%) of ATZ/BEV treated HCC patients exhibit SD or PD, according to mRECIST criteria, and data concerning the clinical outcome of these patients from real life studies are scarce. The aim of our study was to retrospectively evaluate overall survival (OS) of these patients treated under real life conditions with ATZ/BEV combination therapy.

Method: Sixty-nine (69) patients with histological documented unresectable advanced HCC treated with ATZ/BEV combination therapy from January 2020 to September 2024 were evaluated at baseline and every 3 cycles of therapy. Four patients had not been evaluated for response at the time of data cut-off, 15 patients presented complete (CR, n = 2) or partial (PR, n = 13) response
whereas 16 patients exhibited SD and 34 PD, according to mRECIST criteria.

Results: On October 2024, among 50 patients without treatment response (39 males, median age 68 years, 23 of viral etiology, 15 with varices, 11/20/19 with CRAFITY score 0/1/2 and 19/27/4 with ALBI grade I/II/III respectively), 30 were dead (8 with SD and 22 with PD) and 20 were still alive (8 with SD and 12 with PD). Median OS was 3.5 and 5.5 months for dead patients with PD/SD (p = 0.288) whereas it was 9 and 10 months for alive patients with PD/SD, respectively (p = 0.008, compared to dead ones). Alive patients with SD/PD were comparable to dead patients with SD/PD for all baseline parameters evaluated (age, gender, baseline AFP/PIVKA-II/CRP/CRAFITY, presence of varices, histological subtypes) except of ALBI grade (ALBI-I: 13/20 vs 6/30, p = 0.004 respectively). Only ALBI score was significantly different among the two groups after the 3rd (p = 0.037) and 6th (p = 0.05) cycle of therapy as well as the increase in PIVKA-II values after the 6th cycle (p = 0.009). It is important to note that median PIVKA-II values after the 6th cycle of therapy significantly differed among alive and dead patients with SD (143 vs 19208, p = 0.01) as well as among alive patients with PD compared to dead patients with SD (383 vs 19208, p = 0.006) and as expected among alive with SD compared to dead with PD (143 vs 12218, p = 0.048).

Conclusion: Baseline as well as on treatment ALBI grade could predict clinical outcome among non-responder patients with advanced HCC treated with ATZ/BEV therapy. SD/PD according to mRECIST criteria should be possibly re-evaluated taking into account PIVKA-II values during treatment to accurately predict clinical outcomes.

PO5-03
Delta hepatitis versus HBV monoinfection associated hepatocellular carcinoma: spot the difference

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Background and aims: Hepatitis delta virus (HDV) was recently proved to be directly carcinogenic on the hepatocytes, via different mechanisms compared to HBV, leading to a different hepatocellular carcinoma (HCC) pattern. Hence, our aim is to describe the prevalence of HCC in HDV infection versus HBV monoinfection and to highlight the differences between HCC behaviour in both groups.

Method: A retrospective study was conducted in a Hepatology Tertiary Care Centre. All HBsAg positive adult patients admitted from 1st of January 2021 to 31st of December 2023 were included. Statistics was performed using IBM SPSS 29.0. Patients were split in study group: HBV+HDV+HCC and control group: HBV+HCC.

Results: A total of 679 patients were included. The estimated prevalence of HCC in HDV infected population was 20.8 % versus 9.1 % in the control group, p = 0.000, with OR = 2.263, CI 1.536 - 3.333, p = 0.001. Younger patients were found to develop HCC in delta hepatitis (mean ± stdev, 59 ± 8.727 years vs 63 ± 11.28 years, p = 0.027). Patients in the study group have smaller tumors (maximum diameter: 32.66 ± 23.181mm vs 56.75 ± 38.09mm, p = 0.002), but with no difference in AFP values (177.24 ± 364.8ng/ml vs 183.07 ± 336.77ng/ml, p = 0.941) compared to the control group at HCC diagnosis. BCLC classification (p = 0.001) and AFP Duvoux score (p = 0.001) showed more advanced HCC in HBV monoinfection. Hence, treatment in the study group was predominantly loco-regional whereas in the control group was mainly systemic (p < 0.000). The presence of HCC in HDV infected patients was strongly correlated with advanced liver disease (measured by MELD, MELD Na and MELD 3.0, p = 0.001), higher HBsAg titre (p = 0.001) and lower HBV DNA viral load (p = 0.001).

Conclusion: HCC is more frequent in HDV infected patients, leading to a different HCC pattern, with smaller tumours, less advanced neoplasia and access to curative treatment, compared to HBV monoinfection associated HCC. HDV associated HCC occurs in patients with advanced liver disease, higher HBs Ag titre and lower HBV DNA viral load.

PO5-05

LEN-New FP as a promising second-line therapy for patients with unresectable hepatocellular carcinoma refractory to atezolizumab + bevacizumab, durvalumab + tremelimumab, and Lenvatinib

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Background and aims: The 2021 LEOPARD trial showed that hepatic arterial infusion chemotherapy (HAIC) with lenvatinib (LEN) plus a single cisplatin (CDDP) infusion was well tolerated and yielded a high objective response rate (ORR) and a prolonged overall survival (OS) in patients with unresectable hepatocellular carcinoma (HCC). Among HAIC regimens, the New FP regimen, which combines CDDP with lipiodol and continuous 5-fluorouracil (5FU) infusion, has shown a high response rate. Therefore, the combination of LEN and New FP (LEN-New FP) may offer a promising treatment approach. Based on this premise, we implemented LEN-New FP at our hospital and analysed the data of patients receiving second- or later-line therapy following treatment with atezolizumab + bevacizumab (ATZ+BV), durvalumab + tremelimumab (DUR+TRE), or LEN monotherapy.

Method: As of the time of this abstract, 11 patients treated with LEN-New FP for unresectable HCC at our hospital since April 2022 were included. Efficacy was assessed using the modified Response Evaluation Criteria in Solid Tumors, and safety was assessed according to the Common Terminology Criteria for Adverse Events (version 5.0).

Results: Of the 11 patients, nine were classified as NonB-NonC with modified Albumin-Bilirubin (mALBI) grades 1-2b, and ten had a history of prior systemic chemotherapy with ATZ+BV or DUR+TRE. Nine patients exhibited one of the poor OS factors for New FP alone, including a maximum tumor diameter exceeding 7 cm or multifocal distribution across both lobes. Five (45%) were successfully down-staged to a resectable state, and four underwent conversion resection or radical radiofrequency ablation. Among the five patients who achieved down-staging, four had multiple lesions in one lobe or severe vascular invasion without extrahepatic metastases. ORR and disease control rate (DCR) for all patients were 91% and 100%, respectively, with two cases achieving a complete response and four discontinuing due to adverse events. Three patients (27%) had previously received LEN monotherapy, with both ORR and DCR reaching 100%.

Conclusion: With the rapid advancements in the treatment of advanced HCC, treatment targets are continually evolving to achieve drug-free and tumor-free states in select patients with unresectable HCCs. This study found that LEN-New FP was well tolerated, exhibiting extremely high ORR and DCR among patients who received second- or later-line systemic chemotherapy. Despite long-standing debates regarding post-ATZ+BV, DUR+TRE, and LEN therapy for HCC, a definitive treatment strategy is still lacking; however, LEN-New FP may represent a potent treatment option.

PO5-07
The efficacy of treatment for hepatocellular carcinoma in elderly patients

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Background and aims: Despite the increasing proportion of elderly patients with hepatocellular carcinoma (HCC) over time, treatment efficacy in this population is not well established.

Method: Data collected from the Korean Primary Liver Cancer Registry, a representative cohort of patients newly diagnosed with HCC in Korea between 2008 and 2017, were analyzed. Overall survival (OS) according to tumor stage and treatment modality was compared between elderly and non-elderly patients with HCC.

Results: Among 15,186 study patients, 5,829 (38.4%) were elderly. A larger proportion of elderly patients did not receive any treatment for HCC than non-elderly patients (25.2% vs. 16.7%). However, OS was significantly better in elderly patients who received treatment compared to those who did not (median, 38.6 vs. 22.3 months; P<0.001). In early-stage HCC, surgery yielded significantly lower OS in elderly patients compared to non-elderly patients (median, 97.4 vs. 138.0 months; P<0.001), however, local ablation (median, 82.2 vs. 105.5 months) and transarterial therapy (median, 42.6 vs. 56.9 months) each provided comparable OS between the two groups after inverse probability of treatment weighting (IPTW) analysis (all P>0.05). After IPTW, in intermediate-stage HCC, surgery (median, 66.0 vs. 90.3 months) and transarterial therapy (median, 36.5 vs. 37.2 months), and in advanced-stage HCC, transarterial (median, 25.3 vs. 26.3 months) and systemic therapy (median, 25.3 vs. 26.3 months) yielded comparable OS between the elderly and non-elderly HCC patients (all P>0.05).

Conclusion: Personalized treatments tailored to individual patients can improve the prognosis of elderly patients with HCC to a level comparable to that of non-elderly patients.

PO5-09-YI

Early hepatic decompensation identifies patients with hepatocellular carcinoma treated with Atezolizumab plus Bevacizumab or Sorafenib at highest risk of death

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Background and aims: The prognosis of patients with unresectable hepatocellular carcinoma (uHCC) and compensated cirrhosis is influenced by cancer progression. Data on the incidence and the prognostic role of clinical hepatic decompensation following immune checkpoint inhibitor therapy are lacking. We aimed to assess whether early clinical hepatic decompensation (CHD) within 3 months from commencement of systemic therapy affects overall survival (OS) of patients treated with Atezolizumab plus Bevacizumab or Sorafenib

Method: Individual patient data from IMbrave150 trial were analyzed through Vivli platform. Cumulative incidence of CHD was assessed by competing risks analysis against HCC radiological progression. Early CHD and HCC radiological progression were assessed as predictors of OS by time-dependent Cox model.

Results: The 3- and 12-month rates of CHD were 7% and 12%, respectively, while the 3- and 12-month rates of HCC radiological progression were 23% and 52%. Albumin-bilirubin(ALBI)grade 2 (Sub-distribution hazard ratio[sHR] 1.79, 95%CI 1.01-3.19, p=0.049), INR(sHR 1.97, 95%CI 1.64-2.37, p<0.001) and presence of neoplastic macrovascular invasion (sHR 2.01, 95%CI 1.14-3.54, p=0.020) were independently associated with higher risk of CHD. Early CHD(HR 7.56, 95%CI 4.47-12.8) and early HCC radiological progression(HR 5.92, 95%CI 4.03-8.69), as first events, were independently associated with higher mortality.

Conclusion: This study provides robust evidence that early CHD is associated with the highest risk of death in patients with uHCC undergoing systemic treatment. Within well-compensated participants, ALBI, INR and macrovascular invasion identify a population at higher risk of decompensation. Inclusion of clinical decompensation events in future prospective clinical trials may improve characterization of OS from systemic therapy of HCC.

PO5-10
A tale of two cities: HCC surveillance inequalities in the United Kingdom

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Background and aims: Hepatocellular carcinoma (HCC) represents a significant cause of cancer-related mortality worldwide. Six-monthly ultrasound surveillance for HCC is recommended by professional bodies, but studies suggest fewer than 25% of patients receive surveillance at recommended intervals. We aimed to assess HCC surveillance uptake across a multi-centre cohort and examine factors affecting surveillance attendance.

Method: Electronic healthcare data from Northwest London primary care were used to identify number of patients with chronic liver disease. A retrospective review of patients eligible for HCC surveillance was conducted between March 2021- March 2023. We collected data on attendance at surveillance and socio-demographic factors. Multivariate logistic regression analysis was undertaken to examine factors associated with reduced attendance at surveillance.

Results: 2195 patients were identified as having cirrhosis or chronic liver disease in primary care; 869 patients had at least one surveillance episode. Of these, 16.5% patients had received regular surveillance; 73.1% of patients were in receipt of infrequent surveillance and 10.4% received no surveillance over the study period. On multivariate analysis, low socio-economic status and being under the care of a district general hospital were both associated with reduced attendance. Patients aged 45-75 were less likely to be non-attenders at surveillance.

Conclusion: Regular HCC surveillance attendance is low, with patients of low socio-economic status and under the care of non-specialist centres most at risk of reduced attendance at surveillance. Our findings highlight the urgent need to address low HCC surveillance uptake in the UK.

PO5-11-YI

Somatic copy number alterations in circulating cell-free DNA as a predictive biomarker for hepatocellular carcinoma: insights from a proof-of-concept study

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Background and aims: Despite improvements in hepatocellular carcinoma (HCC) management, its prognosis remains poor. Diagnosis at advanced stages often precludes curative treatment options, and currently available biomarkers (e.g., alpha-fetoprotein (AFP)) offer limited utility in early diagnosis and prognostic stratification. Liquid biopsy has emerged as a promising tool for early HCC detection and prognostic evaluation, and the analysis of circulating cell-free DNA (ccfDNA) hold significant potential as a diagnostic tool. This proof-of-concept study aimed to investigate the potential role of tumor fraction (TF) within ccfDNA as a biomarker in HCC patients.

Method: A total of 60 patients were recruited, including 13 with chronic liver disease (CLD), 24 with cirrhosis, and 23 with HCC. Plasma samples were collected, and ccfDNA was extracted for genomic analysis. TF was calculated by focusing on somatic copy number alterations (SCNAs) within the ccfDNA.

Results: In patients with CLD and cirrhosis (n = 37), circulating tumor DNA (ctDNA) was undetectable with the exception of one cirrhotic patient, who presented a significant TF (17 %) and displayed HCC shortly after. Conversely, 5 out of 22 HCC patients (21.7 %) exhibited detectable ctDNA, with TF levels ranging from 3.0 % to 32.6 %. Patients with higher TF levels were characterized by more aggressive disease features, including elevated AFP levels, larger tumor sizes, multiple tumor nodules, and advanced-stage disease.

Conclusion: Preliminary evidence from this study suggests that the analysis of TF, specifically through the detection of SCNAs, could serve as a promising non-invasive tool for the identification and evaluation of HCC. The innovative approach has the potential to significantly enhance early diagnosis and may also improve prognostic stratification in HCC patients.

PO5-12-YI

Outcomes and safety of DAA treatment in HCV cirrhotic patients treated with Atezolizumab-Bevacizumab for hepatocellular carcinoma

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Background and aims: Hepatitis C virus (HCV) infection is a critical driver of hepatocellular carcinoma (HCC) development and progression. Concurrent HCV treatment during systemic HCC therapy has not shown consistent benefits on survival and disease progression. This study aimed to evaluate safety and efficacy of Direct-Acting Antiviral (DAA) therapy in HCV-related HCC patients undergoing Atezolizumab-Bevacizumab (A/B), analysing its effects on overall survival (OS), time to progression (TTP), progression-free survival (PFS), liver decompensation rates.

Method: A total of 135 patients with HCV-related cirrhosis undergoing A/B treatment for HCC were enrolled from 2021 to 2024 and divided into groups based on HCV treatment status: an "active eradication" group (18 patients) who achieved sustained virological response (SVR) after DAA therapy concurrent with A/B treatment, a "prior eradication" group (95 patients) that reached SVR with DAAs or IFN-based regimens at least six months before starting A/B, and a "no eradication" group (22 patients) who did not obtain SVR at all.

Results: 19 patients received SOF/VEL for 12 weeks achieving a 94.7% SVR rate, mostly due to elevated ALT and AST levels (94.4%). No adverse events related to DAA therapy occurred during the treatment course.
The active eradication group demonstrated a significantly improved median OS that was not reached compared to the no eradication group (NA, 95% CI: 22.8–NA vs 20.0 months, 95% CI: 15.5–NA; p = 0.026). Regarding TTP, the active eradication group showed a median of 41.20 months (95% CI: 18.6–NA) compared to 21.3 months (95% CI: 5.13–NA) in no eradication group (p = 0.008). PFS results further supported the benefits of active eradication, with a median PFS of 41.17 months (95% CI: 22.80–NA) compared to 7.76 months (95% CI: 4.53–NA) in the no eradication group (p = 0.012). In contrast, the prior eradication group did not show significant survival benefits compared to the no eradication group for all outcomes. Liver decompensation rates did not differ significantly among groups (p > 0.05), but episodes in the active eradication group occurred only during treatment and the month after completion.

Conclusion: DAA therapy was safe and effective in patients with unresectable HCC receiving A/B treatment. DAA therapy during A/B significantly improved OS, TTP, and PFS in patients with HCV-related HCC, likely enhancing the A/B treatment effect through immunomodulatory mechanisms. This is particularly relevant in settings where maximizing disease control is critical, such as downstaging in patients undergoing liver transplantation.

PO5-13-YI
Prognostic role of serum Glypican-3 measurement in patients with hepatocellular carcinoma

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Background and aims: The clinical management of patients with hepatocellular carcinoma (HCC) is based on a multidisciplinary approach and prognostic algorithms such as the Barcelona Clinic Liver Cancer (BCLC) staging system. However, no biomarker able to reflect tumor biology and behaviour is currently endorsed by clinical practice guidelines. Glypican-3 (GPC-3) is a heparan-sulphate proteoglycan overexpressed in hepatocellular carcinoma (HCC). We aimed to evaluate the prognostic utility of serum GPC-3 measurement in patients with HCC.

Method: A total of 995 consecutive patients with HCC were enrolled between February 2012 and June 2024. Serum samples were collected at HCC diagnosis and stored at -80°C until analyses. Patients with BCLC stage D were excluded from the study. Circulating GPC-3 was measured by ELISA (CanAg Glypican-3 EIA, Fujirebio Diagnostics AB, Sweden). Primary end-point was overall survival (OS).

Results: Overall, 975 patients (median age 66, IQR 58–74 years; 777 [79.7%] males; viral etiology: n = 662 [67.9%]) were included in the study. Most patients had early stage HCC (BCLC 0/A: n = 602; 61.7%) and median OS was 32.1 (95% CI 29.2–36.4) months. Serum GPC-3 values stepwise increased according to BCLC, from 73 (IQR 46–118) pg/mL at stage 0 to 158 (IQR 83–406) pg/mL at stage C ($p < 0.001$). Patients with GPC-3 > 150 pg/mL (Nicolosi et al. 2022) showed lower OS (22.0, 95% CI 18.3–27.4 months) than those with GPC-3 ≤ 150 pg/mL (41.2, 95% CI 34.3–52.6 months) (log-rank test: $p < 0.001$); at multivariate analysis, both GPC-3 > 150 pg/mL (HR = 1.57, 95% CI 1.29–1.91; $p < 0.001$) and BCLC stage (HR = 2.09; 95% CI 1.88–2.33; $p < 0.001$) were significantly and independently associated to OS. Finally, in the subgroup of 73 patients that underwent systemic therapy as first line treatment (BCLC C: n = 49; 67.1%; Sorafenib 72.6%, Lenvatinib 19.2%, Atezo/bev 8.2%), GPC-3 >150 pg/ml resulted significantly associated to reduced survival (HR = 2.70, 95% CI 1.48–4.93; $p = 0.001$) irrespectively from disease control rate at 3 months (HR = 0.25, 95% CI 0.16–0.52; $p < 0.001$) and BCLC stage (HR = 1.75, 95% CI 1.16–2.66; $p = 0.008$).

Conclusion: The assessment of serum GPC-3 may provide prognostic information and contribute to personalized treatment strategies.

Project PNC 0000001 D[3] 4 Health, The National Plan for Complementary Investments to the NRRP, funded by the European Union – NextGenerationEU.

PO5-18-YI
Predictors of esophago-gastric varices and variceal bleeding in patients receiving atezolizumab/bevacizumab for unresectable hepatocellular carcinoma

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Background and aims: Atezolizumab/bevacizumab (atezo/bev) is a standard treatment for unresectable hepatocellular carcinoma (HCC). Bevacizumab may increase the risk of bleeding, causing concerns of variceal bleeding in patients with cirrhosis.
Assess the predictors of esofago-gastric varices, high-risk varices (according to the Baveno criteria), and variceal bleeding in a population receiving atezo/bev for HCC.

Method: Analysis of prospectively collected data from 15 Italian centers included in the ARTE database (March 2022-June2024). Logistic regressions were run for predictors of varices amongst patients who had received an upper-gastrointestinal endoscopy (UGE)<6 months before starting treatment. Competing-risk analyses were performed to assess the predictors of variceal bleeding.

Results: Among 317 patients treated with atezo/bev included in the ARTE database, 256 had a recently performed UGE and were considered for this study. The main characteristcs of the study population were: median age 70 years, 83% male, 58% viral etiology, 88% Child-Pugh A, 52% ALBI-grade 1, 34.3% neoplastic portal vein thrombosis (nPVT), 59.9% Barcelona Clinic Liver Cancer-C stage. At treatment start, 27.3% of patients were receiving non-selective beta-blockers, and 5.8% had received a prior elastica band ligation.
The prevalence of any-type and high-risk varices was 32.0 and 8.6%, respectively. Independent predictors of varices were: platelet count <150,000/mmc (OR 3.69, 95%CI 2.06-6.61), ALBI grade >1 (OR 1.95, 95%CI 1.09-3.48), and nPVT (OR 1.78, 95%CI 1.01-3.18). High risk varices were independently associated with platelet count <150,000 (OR 5.81, 95%CI 1.91-17.67) and ALBI grade >1 (OR 2.44, 95% CI 1.02-5.77). Nine patients had variceal bleeding during the follow-up (G3: n=6; G4: n=2; G5: n=1), accounting for a 3.5% 12-month cumulative incidence. Amongst patients with varices, high-risk varices were the only factor associated with bleeding (sHR 4.06, 95% CI 1.14-14.46) at the competing risk analysis. In these patients the 12-month risk was 12.7%.

Conclusion: The risk of variceal bleeding was low, but non negligible in the subgroup of patients with high-risk varices at baseline. UGE should be performed in all patients before starting treatment: patients with platelet count <150,000/mmc, ALBI grade >1 or neoplastic portal vein thrombosis are at increased risk of varices.

PO6-01
The study on effect of maternal obesity and trained immunity on the development of NAFLD in offspring

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Background and aims: Nonalcoholic fatty liver disease (NAFLD) is increasingly prevalent worldwide, with genetic, epigenetic, and environmental factors contributing to its onset, especially in familial and pediatric cases. This study examines the impact of maternal obesity-induced trained immunity on liver fat deposition in offspring, focusing on the mechanisms underlying intergenerational transmission of metabolic dysfunction.

Method: In this murine study, pregnant mice were divided into two groups: a high-fat diet (HFD) group to induce obesity and a control group on a standard diet. Post-birth, offspring were weaned onto either an HFD or standard diet, creating various combinations to assess the effects of maternal and postweaning diets. Liver samples from the offspring were analyzed for fat accumulation, histological changes, and metabolic markers. Epigenetic modifications, including DNA methylation and histone acetylation (notably H3 acetylation markers), were also examined to evaluate trained immunity transmission. Cytokine profiles and immune cell reprogramming markers were assessed to identify immune modulation in the offspring.

Results: The offspring of HFD-fed mothers exhibited increased liver fat accumulation, heightened macrophage infiltration, and histone acetylation alterations, indicating metabolic reprogramming. Specifically, histone H3 acetylation markers were enriched in HFD offspring, suggesting an epigenetic mechanism underlying trained immunity and metabolic susceptibility. Immune profiling revealed changes in cytokine expression patterns, correlating with liver fat deposition and inflammation.

Conclusion: Maternal HFD and trained immunity significantly impact offspring liver fat deposition and metabolic programming. These findings underscore the role of maternal nutrition in shaping offspring susceptibility to metabolic diseases, particularly NAFLD, through epigenetic and immune-mediated pathways. Understanding these intergenerational mechanisms may aid in developing preventive strategies for NAFLD and related metabolic disorders.

PO6-04
Added-value of positron emission tomography imaging to alpha-fetoprotein score in predicting hepatocellular carcinoma recurrence after liver transplantation

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Background and aims: Despite the increasing use of positron emission tomography (PET) imaging in hepatocellular carcinoma (HCC) context, its utility in predicting recurrence post-liver transplantation (LT) is not clear, and PET is not considered among the selection criteria in LT for HCC. We investigated the correlation between HCC recurrence after LT and PET imaging characteristics before LT.

Method: We retrospectively analyzed 346 patients consecutively transplanted for HCC in Hepato-Biliary Center, Paul Brousse Hospital, between January 2017 and December 2023. Among them, 99 patients had available data before LT on 18F-fluorodeoxyglucose PET (FDG PET) and/or choline PET imaging and were included in the study. We collected pre-LT demographic data, characteristics of liver cirrhosis, HCC and PET imaging and post-LT data. At listing, all patients had an alpha-fetoprotein (AFP) score ≤ 2, according to French selection criteria for LT in HCC. Statistical analyses were performed using STATA 15.1 software and SPSS.

Results: The median age of patients was 63 years and 83 were males (83.8%). The main causes of liver cirrhosis were: viral hepatitis (41.4%), alcohol consumption (39.4%), and metabolic (14.1%). The median MELD (Model for End-Stage Liver Disease) score was 12. Most patients (89.9%) had a bridging treatment while waiting for LT. The median waiting time on waiting list was 5.1 months. The median follow-up post LT was of 36 months. 29 patients (29.3%) experienced HCC recurrence post-LT, with a median time to recurrence of 11.6 months post-LT. The 5-year disease-free survival (DFS) was of 57%, and the 5-year overall survival of 66%. Among the 99 patients with available PET imaging data, 44 patients underwent PET imaging before and after bridging therapy. 102 FDG-PET and 78 choline-PET were analysed, of which 27% of FDG-PET and 35% of choline-PET were positive (intrahepatic only), respectively. In these patients, the complete metabolic response to bridging treatment was associated with a better recurrence-free survival post LT ($p = 0.047$). Net reclassement index, based on AFP score and PET imaging at listing, was of 0.51, $p = 0.02$. We created a nomogram predicting HCC recurrence risk at listing, including PET features.

Conclusion: In our cohort, the recurrence-free survival after LT in HCC patients is better predicted when adding the PET imaging data to AFP score. We need to validate these data in external cohorts, but this could be an indication of a potential role for PET imaging in better selecting the best HCC candidates for LT.

PO6-05
Prognostic factors for survival in patients with intermediate-stage unresectable hepatocellular carcinoma treated with lenvatinib or atezolizumab plus bevacizumab

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Background and aims: Transarterial chemoembolization (TACE) was the major treatment for intermediate-stage hepatocellular carcinoma (HCC) in previous guidelines. Recently, however, the concept of “TACE refractory” and “TACE unsuitable” have established, highlighting the potential of systemic therapy for BCLC stage B HCC. This study explores the prognostic factors for survival in patients with BCLC stage B HCC treated with lenvatinib（LEN）or atezolizumab plus bevacizumab (AB) in real-world setting.

Method: Our study included 155 patients with BCLC stage B HCC who received LEN (n=66) or AB (n=89) treatments at our hospital. We retrospectively analyzed overall survival (OS) and progression-free survival (PFS) and their predicting factors using the Kaplan-Meier method and the Cox proportional hazards model.

Results: In the AB group, patients had a median age of 73 years, 74% were male, with median ALBI score of -2.25 and 73% were beyond up to 7 criteria. The median OS and PFS were 20.9 and 7.9 months, respectively. In the LEN group, patients had a median age of 75 years, 82% were male, with median ALBI score of -2.27 and 69 % were beyond up to 7. The median OS and PFS were 22.8 and 6.9 months, respectively. For patients within up to 7 treated with AB, only baseline ALBI score was significantly associated with OS (HR 5.3, 95%CI 1.2-22.9 ,p=0.03). In LEN group within up to 7, there was no significant factor associated with OS. For patients with HCC beyond up to 7, univariate analysis revealed that baseline ALBI score (HR 2.8, 95%CI 1.2-6.2, p=0.01) and creatinine levels (HR 0.1 ,95%CI 0.01-0.8, p=0.03) were significantly associated with OS. However, in multivariate analysis showed no significant factors associated with OS. In the LEN group beyond up to 7, univariate analysis identified that baseline ALBI score, AFP ≥ 400 ng/mL, and neutrophil/lymphocyte ratio (NLR) were significantly associated with OS. In multivariate analysis, baseline ALBI score (HR 5.2, 95%CI 1.5-18.8, p=0.009) and NLR (HR 1.3, 95%CI 1.0-1.6, p=0.049) were significant independent factors. Neither the maximum intrahepatic tumor diameter nor the number of tumors significantly impacted OS across up to 7 criteria in either treatment group.

Conclusion: In patients with BCLC stage B HCC, systemic therapy outcomes were not predicted by the maximum tumor diameter or the number of tumors. For LEN-treated patients with HCC beyond up to 7, biomarkers like NLR and AFP are crucial for optimizing treatment strategies.

PO6-07
Unveiling the Role of ZBED4 in Hepatocellular Carcinoma: Evidence From the Pan-cancer Analysis and Multiple Databases

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Background and aims: Hepatocellular carcinoma (HCC) is a common and deadly malignancy that has proven difficult to treat. Exploring new targets and therapeutic approaches is quite necessary. The role of ZBED4 in cancers was previously unknown.

Method: The Cancer Genome Atlas (TCGA), the Gene Expression Omnibus (GEO), International Cancer Genome Consortium (ICGC) and the Genomics of Drug Sensitivity in Cancer (GDSC) database were used. Multiple web platforms and software were employed, including R, GEPIA2.0, GSCALite, and CancerSEA for data analysis. The multiplex immunofluorescence was conducted using human HCC tissue micorarray.

Results: High ZBED4 expression was found to be related to unfavorable prognosis and immune infiltration in a variety of cancers. ZBED4 was involved in numerous cancer pathways, including ferroptosis regulation in HCC. In HCC, Tregs and neutrophils were more prevalent in tissues with high ZBED4 expression, while CD8+ T cells, activated CD4+ T cells, gamma/delta T cells, and activated NK cells were more abundant in the low ZBED4 expression group. High ZBED4 expression correlates with poorer immune checkpoint blocking (ICB) response, but better therapeutic responses to chemotherapy and most targeted therapy in HCC patients. Furthermore, a multi-gene prognostic signature was established and validated in different HCC cohorts. The multiplex immunofluorescence study confirmed ZBED4 is associated with an unfavorable prognosis, exhibiting a negative correlation with CD8+T cells infiltration.

Conclusion: Our study clarifies the characterization of ZBED4 and its close association with immune infiltration and highlights its potential value as a promising biomarker for prognosis and therapy in HCC.

PO6-08
Durvalumab/Tremelimumab in real life: results of a prospective cohort

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Background and aims: Two immunotherapy combinations are currently recommended for the treatment of locally advanced or metastatic hepatocellular carcinoma (HCC) in 1st line. The combination DURVALUMAB and TREMELINUMAB (DT) has recently been validated, but no real data are available

Method: In 2 hepatology departments, we prospectively included patients prescribed DT immunotherapy between April 2023 and November 2024. We prospectively collected patient characteristics, response to DT, immune-mediated side effects, progression-free survival and overall survival.

Results: Sixty-two patients were included over this period (F/H: 9/53), 85.7% of whom had cirrhosis (Alcohol-Associated Cirrhosis: 56.5%; Metabolic associated fatty liver disease: 30.6%), 90.5% of whom were considered CHILD A. HCC was histologically proven in 56.5% of patients. DT was chosen for problems of portal hypertension (32.3%), cardiovascular history (30.6%), kidney disease (14.5%) or concomitant radiotherapy (9.7%). Half the patients were on anticoagulant or antiaggregant therapy at initiation of DT. Median age at initiation of DT was 73 years (IQR: 65.9-76.7). DT was the first treatment for HCC in 61.3% of patients, 53.2% with BCLC grade C. Median AFP was 41µg/L (IQR: 4.7-810). Median follow-up was 5.3 months (IQR: 2.3-8.6). Thirty-three (52.4%) developed toxicity (any grade) attributed to immunotherapy, with a median delay of 56 days (IQR: 33.2-85.5 days). Eleven grade 3/4 toxicities were identified in 14 patients (17.8%) with a median delay of 37 days: colic (n=5), cardiac (n=4) and hepatic (n=3). Overall survival was 64.7% and 53.0% at 6 and 12 months, respectively. Progression-free survival was 51.7% and 35.6% at 6 and 12 months. The objective response rate according to mRECIST was 36.2%.

Conclusion: This first real-life study confirms the feasibility of this dual immunotherapy combination in a real population. Grade 3/4 toxicities remain limited in our population.

PO6-09
Comparative Survival Analysis of Hepatocellular Carcinoma Patients With and Without Secondary Primary Tumors

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Background and aims: The presence of secondary primary tumor (SPT) in patients with hepatocellular carcinoma (HCC) adds significant complexity to clinical management and prognostic. While HCC is associated with poor survival outcomes, the impact of SPT on overall survival compared to patients with HCC alone remains inadequately explored. This study aims to find the differences between these two patient groups

Method: Data of 537 HCC patients included in a prospective cohort study at the Inselspital, Bern, were retrospectively reviewed. This cohort consisted of 450 patients with HCC only and 87 with both HCC and a secondary primary tumor (SPT). Over an 8-year period, cumulative incidences of death and the development of SPT were assessed using Kaplan-Meier estimates, and risk factors for SPT were evaluated using Cox regression analysis

Results: Patients with both HCC and SPT were significantly older than those with HCC alone (70 vs. 65 years; p=0.007). No statistically significant differences were observed between the groups in terms of gender, comorbidities (including diabetes mellitus, hyperlipidemia, and obesity), or underlying liver disease etiologies (i.e. metabolic-associated steatotic liver disease, alcohol-related liver disease, hepatitis C virus, hepatitis B virus, and other causes). Patients with both HCC and SPT were more likely to have less advanced HCC, classified as BCLC stage A, compared to those with HCC alone (54% vs. 39%, p=0.035). Vascular invasion was more frequent among HCC-only patients than those with SPT (13% vs. 4.7%, p=0.035). The cumulative risk of developing an SPT remained relatively low, increasing from 1.8% at 1 year to 9.6% at 8 years. In contrast, mortality in the overall population rose sharply, from 21.7% at 1 year to 62% at 8 years. No significant difference in survival was found between patients who developed an SPT and those who did not (p=0.128).

Conclusion: SPTs develop in HCC patients, who are generally older and have an HCC in earlier disease stages. The risk of SPT development in HCC population increases gradually over the time and does not seem to significantly impact overall survival.

PO6-10
Association of Hyperglycemia and Outcomes after Hepatectomy for Hepatocellular carcinoma

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Association of Hyperglycemia and Outcomes after Hepatectomy for Hepatocellular carcinoma

Background and aims: Hepatocellular carcinoma (HCC) has been linked to metabolic conditions like metabolic syndrome and diabetes. While blood glucose levels are associated with outcomes in other cancers, their role in HCC recurrence is not well understood. This study investigates whether preoperative blood glucose, independent of diabetes status, is associated with HCC recurrence and survival.

Method: This retrospective study included 81 HCC patients who underwent elective liver resection at Heidelberg University Hospital between 2015 and 2021. Patients were grouped by fasting blood glucose: hyperglycemia (above 125 mg/dl) and normoglycemia (below 100 mg/dl). Statistical analysis was performed using SPSS, employing t-tests and Chi-square tests. The significance level was set at $p < 0.05$.

Results: Recurrence was assessed at 1-, 2-, and 3-years post-surgery. Most recurrences occurred within the first year. Patients with normoglycemia experienced fewer recurrences than those with hyperglycemia: after 3 years, 35 % of patients in the normoglycemia group had a recurrence, compared to 46.3 % in the hyperglycemia group ($p = 0.299$). Additionally, the hyperglycemia group showed a higher mortality rate of 17.1 %, compared to 5.0 % in the normoglycemia group, although this difference was not statistically significant ($p = 0.084$). In-hospital mortality rates were similarly low in both groups, with no significant difference ($p = 0.542$).

Conclusion: Normoglycemic patients showing trends toward lower recurrence and mortality compared to hyperglycemic patients. However, these differences were not statistically significant, likely due to the limited sample size. These findings underscore the need for a larger, prospective, multicenter study to clarify blood glucose's role in HCC outcomes. Further, studies into association of hyperglycemia treatment after HCC resection can be proposed.

PO6-11
Statins in patients with advanced HCC treated with atezolizumab/bevacizumab: a propensity score-matched cohort analysis from ARTE an Italian prospective multicentric dataset

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Background and aims: Statins have been suggested to exert anticancer properties by modulating angiogenesis, fibrosis, inflammation, and the tumor microenvironment, generating interest in their clinical use for chronic liver diseases (CLD) and hepatocellular carcinoma (HCC) chemoprevention. However, the effects of statin therapy in patients treated with immune checkpoint inhibitors for CLD-associated HCC remain unknown. This study primarily aimed to assess the potential effect of statins on overall survival (OS) and progression-free survival (PFS) in patients with advanced HCC treated with atezolizumab and bevacizumab (A+B).

Method: The ARTE dataset, a retro-prospectively maintained database, includes 305 consecutive patients with unresectable HCC treated with A+B, enrolled from 12 tertiary care centers in Italy. From the original cohort, a 1:1 propensity score matching was performed to balance potential confounding factors between 63 patients on statin therapy and those who were not. The primary outcomes were OS and PFS, while secondary outcomes included all-cause mortality, liver-related death, treatment interruption, and incidence of liver decompensation events.

Results: Among the matched population of 126 patients, 75% had liver cirrhosis, with metabolic disfunction -associated steatotic liver disease (MASLD) being the most common etiology. Ninety-seven patients (32%) had diabetes. No significant differences were found between statin users and non-users for OS, PFS, or liver-related death. Additionally, the log-rank test revealed no significant difference between the groups in terms of treatment interruption due to liver decompensation events (p=0.28).

Conclusion: Statin use did not show any impact on OS, PFS, or reduction in mortality or treatment interruption due to liver-related decompensation events in patients with advanced HCC treated with A+B.

PO6-12
Immune checkpoint inhibitors as adjunctive therapy with locoregional treatment in patients with advanced hepatocellular carcinoma is tolerable and improves disease-free survival: real-world experience

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Background and aims: The Global Disease Burden of Hepatocellular Carcinoma (HCC) is well documented and worsening due to the increase in HCC incidence. Although liver transplantation is considered curative therapy, a significant number of HCC cases are identified beyond MILAN criteria. Various Immune Checkpoint Inhibitors (ICI) have been recently approved as a treatment of advanced unresectable HCC. Locoregional treatment (LRT) is used to manage HCC. We report on the use of complementing LRTs with ICIs in patients with HCC in a non-academic, community-based single Liver center.

Method: Between May 2018 and October 2024, 451 HCC patients (pts) were seen at a liver cancer clinic. 281 pts were not prescribed ICI (not indicated, contraindicated, or pts had metastatic cancer). 170 pts were prescribed ICI as adjunctive therapy to LRT (primarily TACE, RFA or Y-90). 46 did not start ICI because of insurance denials, pt refusal, or referral to hospice. 125 pts initiated treatment with ICI. Of those, 100 received at least 2 doses and are the subjects of this analysis. ICI was infused per the package insert and pts were followed per standard of care. Labs, imaging, further locoregional interventions, disease progression, transplantation candidacy, and survival were analyzed.

Results: 82.4% were males. 69.8% Hispanic. Mean age was 64.8 ± 7.6 years. All were Child-Pugh A or B. Almost 60% of pts were BCLC A3 or B prior to ICI start. ICI treatment: 52% were treated with nivolumab (nivo) only, 26% with nivolumab/ipilimumab, 13% with atezolizumab/bevacizumab (ate/bev), and 9% with durvalumab/tremelimumab. Median duration on ICI was 15 (1-58) months. Safety and Tolerability: Liver enzymes and MELD score were stable throughout treatment. Pts experienced manageable side effects such as nausea, vomiting, and fatigue. ICI/LRT outcome: HCC recurrence was 12%. Liver transplant 8%. 12% became MILAN eligible post ICI. There was a statistically significant decrease in the average number of viable lesions and average size of viable lesions post ICI treatment. Survival: 78.5% alive. Median survival from HCC diagnosis was over 4 years and 2 years with HCC-free survival.

Conclusion: The most tolerated ICI regimen with the least discontinuations was nivolumab only followed by nivolumab/ipilimumab. Atezolizumab/bevacizumab regimen was the least tolerated. In patients with advanced HCC managed by a community-based single Liver Center, the combination of LRT and ICI was: 1) safe and well-tolerated, 2) more effective than LRT alone with fewer need of LRT, and 3) prolongs disease stability and overall transplant-free survival.

PO6-13
18F-fluorodeoxyglucose positron emission tomography coupled with computed tomography (18F-FDG PET/CT) as a recurrence prognosis tool in liver transplantation for hepatocellular carcinoma

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Background and aims: In liver transplantation (LT) for hepatocellular carcinoma (HCC) there is a clear need for better prognostic factors than size and number of nodules. The aim of this study was to evaluate the prognostic value of 18F-fluorodeoxyglucose positron emission tomography coupled with computed tomography (18F-FDG PET/CT) in preLT assessment of patients with chronic liver disease and HCC.

Method: This is a single-center retrospective study on 115 HCC patients (mean age: 62 ± 7 y) who benefited from 18F-FDG PET/CT before any bridge therapy and underwent LT between January 2010 and December 2021 at Liege University Hospital. Follow-up was ended in December 31, 2023. 18F-FDG PET/CT was considered positive when the ratio between the SUVmax of the tumor and the SUVmax of the liver parenchyma (RSUVmax) was > 1.15 (PET pos). RSUVmax was compared to other preLT prognostic factors, as Milan criteria (in/out), AFP score (≤ or > 2), Up-To-Seven score (in/out, and Metroticket 2.0 score. Overall survival and recurrence-free survivals were evaluated by Kaplan-Meier method. Comparisons of survival between prognostic factors were made using Cox regression.

Results: Among the 115 HCC patients, 32 patients (27.8%) were Milan out, 21 (18.3%) AFP score >2, and 11 (9.6%) Up-to-seven out at time of LT listing. RSUV max was >1.15 in 28 (24.7%) patients. Overall survival was 88.7% and 70.8% at 2 and 5 years, respectively. Recurrence-free survival (RFS) was 91.6% and 80.8% at 2 and 5 years, respectively. In recurrence-free survival analysis in the Milan out group, RSUVmax > 1.15 was the only predictive factor for recurrence in uni- and multivariate analyses. Interestingly, there was no 5-yr RFS difference between Milan in/PET neg, Milan in/PET pos and Milan out/PET neg patients (88%, 83% and 77% respectively), but RFS was significantly worse in Milan out/PET pos patients (20%).

Conclusion: FDG PET/CT with an RSUVmax cut-off value of 1.15 is a strong prognostic factor for recurrence in Milan outpatients. Further prospective studies should test whether 18F-FDG PET/CT) should be systematically included in the preLT assessment.

PO6-14
Clinical Characteristics and Outcomes for Hepatocellular Carcinoma with Extrahepatic Metastases

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Background and aims: Hepatocellular carcinoma (HCC) is a significant health concern in Taiwan, ranking fifth in incidence and second in cancer-related deaths. HCC with extrahepatic metastases (EHM) impacted overall survival and systemic therapies were recommended by several guidelines. The role of aggressive locoregional control for advanced-stage HCC with EHM remains controversial.

Method: An analysis of 4,161 HCC patients admitted to Kaohsiung Veterans General Hospital between 2005 and 2022 were performed and 552 patients with EHM at initial diagnosis were identified. Clinical characteristics and outcomes were analyzed.

Results: EHM was detected in 552 (13.3%) of patients. Metastases to lymph nodes in 266 (48.2%), lungs in 182 (33%), bones in 61 (11%), adrenal glands in 34 (6.2%), and other sites in 8 (1.4%) patients were found. Various treatments were administered, including locoregional, systemic, and combined locoregional and systemic treatments and palliative treatment. Multivariate analyses revealed alcohol use, tumor numbers more than 2, tumor size greater than 5 cm, and portal vein invasion as significant risk factors for developing EHM. Patients without EHM had a significant longer median survival than those with EHM (24 Vs. 3 months, $p<0.001$). In HCC patients with EHM, tumor numbers more than 2, tumor size greater than 5 cm, and portal vein invasion were independent risk factors for survival. In patients with EHM who received systemic treatments had longer survival (6 Vs. 2 months, $P=0.016$). In EHM patients who received systemic therapy, combined locoregional therapy demonstrated a significant survival benefit (8 Vs. 3 months, $p<0.001$).

Conclusion: This study disclosed the clinical features and prognostic factors of HCC with EHM. In EHM patients who received systemic therapy, combined locoregional therapy demonstrated a significant survival benefit

PO6-15
Real-world experiences of cabozantinib after immunotherapy in patients with unresectable HCC

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Background and aims: Cabozantinib (CAB) has been widely used after immune checkpoint inhibitors (ICI) in patients with unresectable hepatocellular carcinoma (u-HCC). In the phase 3 trial of CAB plus atezolizumab, CAB was administered at 40mg/day in CAB plus atezolizumab group. We investigated the impact of the initial dose and clinical outcome of CAB after ICI in real-world practice.

Method: A total of 55 u-HCC patients who received CAB after ICI between Jan 2021 and Aug 2024 at our institution was enrolled. Tumor assessments in accordance with RECIST ver1.1 were done using dynamic CT or MRI within 4-8 weeks and every 8-10 weeks thereafter. Adverse events (AEs) were reported according to CTCAEv5.0. The initial dose of CAB was decided based on the patient's condition. All clinical data were analysed retrospectively.

Results: The median age was 75 years, 32 patients were without hepatitis virus infection, and 43 patients were Child-Pugh A. BCLC stage A/B/C were 2/ 21/ 32 patients, and 54 patients were previously treated with atezolizumab plus bevacizumab. One patient received durvalumab plus tremelimumab before CAB. Two immune-based combination therapies were performed before CAB in 4 patients. CAB was introduced as 2nd (n=9), 3rd (n=24), 4th (n=13), 5th (n=4), 6th (n=4), and 7th -line (n=1). The median OS and PFS in Child-Pugh A patients were 4.1 and 19.1 months, while they were 3.0 and 8.5 months in Child-Pugh B patients. The objective response rate (ORR) and disease control rate (DCR) were 11.6 and 79.1 %. The initial dose of CAB was 60mg/day (n=12), 40mg/day (n=17), 20mg/day (n=19), 20mg every other day (n=7). The full dose induction (60mg/day) was not a significant factor associated with PFS and OS. The median relative dose intensity (RDI) of CAB for the first month (1M-RDI) was 30.3%, and there were no significant differences in PFS and OS between the patients with $\geq$ 30% 1M-RDI (n=20) and < 30 % 1M-RDI (n=23). Adverse events (AEs) were observed in all patients, requiring dose reduction in 32 patients, and interruption was reported in 30 patients. The induction rate of molecular targeted therapies after CAB was 57.5%.

Conclusion: The OS of CAB after ICI was better than the phase III CELESTIAL trial. The full dose induction (60mg/day) and maintaining a high 1M-RDI would not be necessary for CAB treatment after ICI. Modifying the daily dose and appropriately interrupting CAB are essential in real-world practice.

PO6-16
Evaluation of epidemiological trends and recurrence rates in unresectable hepatocellular carcinoma treated with microwave ablation: a 14-year study

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Background and aims: Incidence and mortality of hepatocellular carcinoma (HCC) is globally increasing due to rising metabolic-associated steatotic liver disease (MASLD), while hepatitis B and C infections and alcohol-related liver disease (ALD) remain key risk factors. For patients ineligible for resection or transplant, microwave ablation (MWA) offers a safe and effective minimally invasive option. This study assesses changes over 14 years in the epidemiology and recurrence rates of HCC following MWA, focusing on three time periods: 2010-2014, 2015-2019, and 2020-2023.

Methods: A retrospective analysis was conducted on 287 HCC patients treated with MWA at a single center (Garibaldi-Nesima Hospital, Catania, Italy). US-guided procedures were performed by a single operator, used the Amica System™ (HS, Italy), with parameters including 2450 MHz frequency, ablation power 60-140W, and exposure times 3-10minutes, guided by tumor size and manufacturers' specification. All contrast-enhanced CT or MRI evaluations were performed by three expert radiologists. Six-month recurrence rates were stratified by tumor size (<2 cm, 2-3 cm, >3 cm). We analyzed changes in patient demographics, underlying liver disease, and HCC size distribution across the study periods.

Results: Over the study period, HCC cases driven by MASLD increased, while HCV-related cases declined, aligning with global trends. The proportion of female patients grew from 32% in 2010-2014 to 40% in 2020-2024, reflecting demographic shifts in metabolic disease. Recurrence rates dropped notably, from 55% in 2010-2014 to 33% in 2020-2023. Tumor size analysis revealed changing recurrence patterns, with 2020-2024 showing the lowest recurrence for tumors <2 cm (14%) versus higher rates for 2-3 cm (36%) and >3 cm (20%). Interestingly, in the most recent four-year period, tumors sized between 3 and 6.5 cm exhibited a notably low recurrence rate (20%), underscoring the efficacy of MWA for larger tumors. Tumors <2 cm made up 60% of cases by 2020-2024, possibly due to improved early detection and surveillance practices.

Conclusion: This 14-year study shows a change patient demographics and HCC risk profiles and a marked improvement in six-month recurrence rates after MWA, especially for smaller tumors. MWA is effective even for larger tumors. The findings underscore the need for updated screening and tailored intervention strategies that incorporate metabolic risks.

PO6-18
TACE combined with ICIs plus MTT after 125I irradiation stent placement in patients with hepatocellular carcinoma and main portal vein tumor thrombosis (PATENCY Ⅱ)

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Background and aims: Patients with hepatocellular carcinoma (HCC) and main trunk (Vp4) portal vein tumor thrombosis (PVTT) have a poor prognosis, and current treatment options provide limited benefits. We aimed to assess the safety and efficacy of transcatheter arterial chemoembolization (TACE) combined with immune checkpoint inhibitors (ICIs) plus molecular targeted therapy (MTT) after irradiation stent placement (ISP) as first-line treatment for these patients.

Method: This multicenter retrospective cohort study enrolled 444 patients with HCC and Vp4 PVTT treated with either ISP, TACE, ICIs, and MTT (ISP-containing quadruple group, n = 131) or with ICIs and MTT (ICIs-MTT group, n = 313) between January 2020 and May 2023. Propensity score matching was used to balance the groups. The primary endpoint was overall survival (OS). The secondary endpoints included progression-free survival (PFS), objective response rate (ORR), PVTT response, patency of portal vein, and safety.

Results: After propensity score matching (1:2), 127 patients from the ISP-containing quadruple group were matched with 220 patients from the ICIs-MTT group. The median OS (10.3 months, interquartile range [IQR]: 9.1-11.5 vs 8.6 months, IQR: 7.6-9.6; $P = 0.004$), PFS (6.1 months, IQR: 4.9-7.3 vs 3.5 months, IQR: 2.9-4.1; $P < 0.001$), and ORR (52.8% vs 27.7% with RECIST version 1.1; 58.3% vs 29.1% with mRECIST) were higher in the ISP-containing quadruple group than in the ICIs-MTT group. The ISP-containing quadruple group also demonstrated a higher PVTT positive response rate (64.6%) than the ICIs-MTT group (20.5%). Median stent patency was 10.4 months (IQR: 8.2-12.7). Grade ≥3 adverse events were observed in 37 patients (29.1%) in the ISP-containing quadruple group and 56 patients (25.5%) in the ICIs-MTT group ($P = 0.456$).

Conclusion: Following ISP, treatment combining TACE with ICIs plus MTT can significantly prolong OS and PFS in patients with HCC and Vp4 PVTT and is generally well-tolerated.

PO6-19
The ALBI score as a tool to optimise allocation to immunotherapy in patients with hepatocellular carcinoma: insights from a multicentre cohort

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Background and aims: Liver function is essential in selecting hepatocellular carcinoma (HCC) patients for immunotherapy. The Child-Pugh score is widely used for assessing liver function in cirrhotic patients, but it has limitations, such as the subjective evaluation of ascites and hepatic encephalopathy. Furthermore, the Child-Pugh B category is heterogeneous, complicating treatment decisions. The modified Albumin-Bilirubin (mALBI) score provides a more objective and reliable assessment. This study evaluates whether the mALBI score better assesses liver function in HCC patients treated with atezolizumab and bevacizumab.

Method: Retrospective analysis of 101 HCC patients treated with atezolizumab and bevacizumab across seven Swiss centers. Patients were categorized by mALBI grades (1, 2a, 2b, and 3), and outcomes included objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), and overall survival (OS). Statistical significance was determined at $p < 0.05$.

Results: Median age was 67 years (IQR 60–73), 76% male and 82% cirrhotic. Patients with mALBI grade 1 (n = 36) had a median OS of 23 months, compared to 14 months for grade 2a, 15 months for grade 2b, and 4.9 months for grade 3 ($p = 0.015$). Grade 1 patients had higher ORR (41%) and DCR (12%) than those with grades 2a, 2b, and 3 ($p = 0.04$). Median PFS was 12 months for grade 1, significantly longer than for grades 2a (5.7 months), 2b (4.5 months), and 3 (4.2 months) ($p = 0.06$). Among Child-Pugh A patients (n = 65), median OS was 20 months, compared to 4.5 months in Child-Pugh B patients (n = 13). Within the Child-Pugh A group, grade 1 patients had a median OS of 23 months, while grades 2a and 2b had OS values of 17 and 13 months, respectively ($p > 0.044$). PFS was also longer for grade 1 (14 months) compared to grades 2b (6.8 months) and 3 (4.6 months) ($p < 0.01$). In Child-Pugh B patients, grade 2a had an OS of 10 months, compared to 4.9 months for grade 2b and 4.8 months for grade 3 ($p > 0.9$). PFS for grade 2a was 10 months, compared to 4.2 months for grade 2b and 3.4 months for grade 3 ($p > 0.9$).

Conclusion: The mALBI score appears to be a more reliable tool for assessing liver function and could refine patient selection for atezolizumab and bevacizumab therapy, particularly for Child-Pugh B patients. Larger studies are needed to validate these findings.

PO6-20
Updated Network Meta-Analysis of First-Line Systemic Therapies for Advanced Hepatocellular Carcinoma: Consistent Role of TACE

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Background and aims: We conducted an updated network meta-analysis to evaluate and identify the optimal first-line treatment for advanced hepatocellular carcinoma (HCC) among all relevant transarterial and targeted therapies.

Method: We analyzed 25 phase 2 or 3 randomized controlled trials involving 14,148 patients with metastatic or unresectable HCC between 2008 and 2024, which evaluated 20 systemic agents and 4 transarterial interventions combined with systemic therapy, using sorafenib or lenvatinib as standard controls. Primary outcome was overall survival (OS), and secondary outcomes included progression-free survival (PFS) and grade 3-4 adverse events. Subgroup analyses were conducted to assess individual treatment efficacies in specific clinical settings.

Results: Transarterial chemoembolization (TACE) combined with lenvatinib provided the greatest improvement in OS compared to sorafenib, with a hazard ratio of 0.41 (95% confidence interval, 0.30–0.58), followed by sintilimab+IBI305 (0.57; 0.43–0.75), camrelizumab+rivoceranib (0.62; 0.48–0.80), atezolizumab+bevacizumab (0.66; 0.51–0.85), lenvatinib+pembrolizumab (0.77; 0.62–0.97), and tremelimumab+durvalumab (0.78; 0.64–0.95). These combinations, except tremelimumab+durvalumab, were also regimens significantly superior to sorafenib regarding PFS. TACE+lenvatinib was ranked first in OS analyses with the other current standard-of-cares (lenvatinib, atezolizumab+bevacizumab, and tremelimumab+durvalumab) as a control. TACE+lenvatinib, sintilimab+IBI305, and atezolizumab+bevacizumab demonstrated consistent significance in extending OS over sorafenib in subsets with portal invasion, extrahepatic metastasis, and hepatitis B. All immunotherapy-based combinations were significantly associated with a higher risk of adverse events than sorafenib.

Conclusion: Our first-line analysis consistently scored TACE+lenvatinib best for survival outcomes, followed by various immunotherapy-based combinations in advanced HCC. This hierarchy was sustained in aggressive tumors or hepatitis B carriers.

PO6-21
The evolving treatment landscape of advanced biliary cancer (ABC): a real-world overview of the last two decades

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Background and aims: The recent advent of genomics-driven precision medicine and immunotherapy-based combinations along with the proven efficacy of second-line chemotherapy have reshaped treatment paradigms in ABC. Whether these advances are favourably impacting disease trajectory and to what extent is currently unknown in clinical practice. We performed a real-world analysis of treatment patterns and survival outcomes of ABC over last 20 years.

Method: Consecutive ABC diagnosed at the University Hospital of Modena from January 2004 to January 2024 were identified. Patients (pts) demographics, disease characteristics, treatment patterns, and clinical outcomes were collected across four 4-year periods: 2004-2009, 2009-2014, 2014-2019 and 2019-2024. Survival curves were estimated and plotted using the Kaplan-Meier method and compared using the log-rank test.

Results: Overall, 381 pts were included: the median age was 69 years, 47% had intrahepatic cholangiocarcinoma and 82.5% presented with metastatic disease. Among them, 335 (88%) pts received at least one line, 164 (49%) two lines and 64 (20%) three lines. The most commonly adopted regimens in first-line were platinum-gemcitabine (45%) and cisplatin, gemcitabine, durvalumab (67%) before and after February 2022, respectively. In the second-line setting, FOLFIRI (32%) was the most frequently administered regimen followed by capecitabine (24%) and FOLFOX (15%). The uptake of second-line increased considerably from the first to the fourth quarter of the study period (from 40% to 55%; p < 001). The median overall survival (OS) was improved over time (p = 0.002) and was correlated with the number of treatment lines received (7.3, 10.7 and 22.1 months for 1, 2 and 3 lines; p < 0.001). The addition of anti-PD(L)-1 to cisplatin and gemcitabine significantly prolonged OS compared to a 1:1 matched cohort of historical controls treated with chemotherapy alone (n=87). Among 129 pts undergoing NGS-based molecular profiling, ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) I-II alterations were found in 23% of cases (including 14 IDH1 mutations, 6 FGFR2 fusions, 4 $BRAF^{V600E}$ mutations, 3 HER2 amplifications, 2 $KRAS^{G12C}$ mutations, 1 MSI-H) with molecularly-matched therapies resulting in a median OS of 9.2 months.

Conclusion: Despite one in ten pts remaining ineligible for active treatment, a growing proportion of ABC has received sequential treatment lines with incremental advantage on survival. Implementing chemo-immunotherapy for all-comers and targeted therapies for molecularly-defined subsets has proven effective in clinical practice. This study depicted evolving standards in the management of ABC thus informing future studies on this topic.

PO6-22
Integrating machine learning and mathematical modeling to predict hepatocellular carcinoma recurrence and optimize outcomes after liver transplantation

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Background and aims: Cancer recurrence remains the leading cause of death following liver transplantation (LT) for hepatocellular carcinoma (HCC). Although various models have been developed to predict recurrence, the application of machine learning and advanced mathematical modelling in this area is still limited. We therefore aimed to develop a machine learning derived model to predict HCC recurrence after transplantation and show its potential to maximize patient survival.

Method: We developed several machine learning classifiers using clinical data from the Swiss Transplant Cohort Study (STCS) of patients transplanted for HCC (n = 505) to predict recurrence. To estimate the clinical impact of our model, we integrated this classifier with a mechanistic mathematical model to optimize survival outcomes. Integrating the classifier into a Monte Carlo simulation approach, we evaluated personalized treatment strategies, quantifying their potential to enhance long-term survival.

Results: The overall HCC recurrence rate after HCC transplantation in the STCS cohort was 14%, the median time to recurrence was 22 months, the mean follow-up time was 42.7 months. The dataset was used to train different machine learning classifiers and Random Forest showed the best performance in predicting HCC recurrence. The following variables were identified to be associated with recurrence: Alpha-Fetoprotein (AFP) before LT, ALAT before LT, Cold Ischemia time, ALBI score before LT, Bilirubin before LT, Donor Age, MELD score, Albumin before LT and Vascular Invasion. Finally, the Monte Carlo simulations suggest that the predictive information from the Random Forest could optimize existing liver transplantation protocols, therefore improving overall survival outcomes.

Conclusion: Our results demonstrate that combining predictive analytics with mechanistic modeling enables a data-driven, individualized HCC recurrence risk prediction. While this approach underscores the potential of advanced modeling techniques to improve patient prognosis in liver transplant oncology, future work involves validating the model in additional datasets.

PO7-01-YI
Impact of MBOAT7 rs641738 polymorphism on prognosis of metabolic dysfunction-associated steatotic liver disease patients with hepatocellular carcinoma

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Background and aims: Several genetic factors have been associated to liver disease onset and progression in patients with metabolic dysfunction-associated steatotic liver disease (MASLD). In particular, genetic variants predisposing to liver fat accumulation (i.e. *PNPLA3*, *TM6SF2*, *MBOAT7*, and *GCKR*) were associated to increased risk of hepatocellular carcinoma (HCC) development. We aimed to investigate the association between genetic variants and prognosis of patients with MASLD-HCC.

Method: A total of 225 MASLD patients (median age: 74, IQR 49–97 years; males: 195, 86.7%) with a diagnosis of HCC were retrospectively enrolled. Most patients had an early stage HCC (BCLC: 0/A, n = 145, 64.4%); patients with BCLC stage D were excluded from the analysis. Genotyping for *PNPLA3* rs738409 (C > G), *MBOAT7* rs641738 (C > T), *TM6SF2* rs58542926 (C > T), *GCKR* rs780094 (C > T) was performed by real-time allelic discrimination assay (TaqMan SNP Genotyping Assay, Applied Biosystems). Primary end-point was overall survival (OS).

Results: Overall, median OS was 29.1 (95% CI 21.4–32.7) months. Among the four genetic variants analysed, only *MBOAT7* rs641738 showed significant results at survival analysis. Specifically, patients who carried the TT risk genotype (n = 59) showed reduced OS compared to those with CC / CT genotype (n = 167) (OS = 19.8, 95% CI 16.4–27.0 months vs. 32.2, 95% CI 28.5–53.9 months, respectively; p = 0.007). At multivariate logistic regression analysis, *MBOAT7* rs641738 TT genotype resulted associated to reduced OS (OR = 1.62, 95% CI 1.04–2.51) independently from BCLC stage (OR = 2.12, 95% CI 1.68–2.68).

Conclusion: *MBOAT7* rs641738 (C > T) variant was associated to poor prognosis in patients with MASLD-HCC. In such patients, *MBOAT7* rs641738 genotyping may be useful to improve clinical management and to support decision-making.
Project PNC 0000001 D[3] 4 Health, The National Plan for Complementary Investments to the NRRP, funded by the European Union – NextGenerationEU.

PO7-04-YI
Exploring the effectiveness of Machine Learning Algorithms in predicting Non-viral Hepatocellular Carcinoma (NVHCC) development: A Multicenter Cross-Sectional Study

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Background and aims: Viral hepatitis B and/or C are the leading causes of hepatocellular carcinoma (HCC). However, with the rising incidence of obesity, metabolic-related disorders are increasingly associated with a higher incidence of non-viral HCC. Machine learning (ML) helps develop predictive models that calculate individual HCC risk and guide selective screening and risk mitigation strategies. This study aimed to build a predictive model of sufficient accuracy for diagnosing non-viral HCC using baseline routine data to estimate the risk of HCC

Method: A retrospective multicenter cohort of 503 patients (100 patient with non-viral liver cirrhosis and 403 patients with non-viral HCC) was recruited from six tertiary care centers in Egypt (Assiut University, Damanhur medical institute, Helwan University, Hepatoma group of Ain-Shams University, Multidisciplinary HCC clinic of Cairo University, Shebein El-kom National liver institute) from March 2007 to June 2022. The dataset includes more than forty individual standard laboratories and clinical parameters. Alternating Decision Tree (ADT), Naïve Bayes Tree (NBT), Random Forests (RF), Logistic Model Trees (LMT) were utilized to accurately diagnose non-viral HCC. A cross-validation with 10-fold was used to avoid over training problems. The Area under Receiver Operating Characteristic curve (AUROC) and accuracy were used to evaluate the performance

Results: Age, Gender, cigarette smoking, Platelets, international normalized ratio (INR), total bilirubin, albumin, and LDL cholesterol were typically found to be significant predictors. Hepatic steatosis index (HIS), Albumin-bilirubin score (ALBI) score, easy-ALBI score (EZ-ALBI), Fibrosis-4 (Fib4), model for end-stage liver disease (MELD) score, CHILD-PUGH score, Platelet albumi bilirubin (PALBI) score, and platelet albumin (PAL) score were the most significant scores. Negative Predictive Value, Positive Predictive Value, Sensitivity, Specificity, Negative Likelihood Ratio (LR-), and Positive Likelihood Ratio (LR+), Accuracy and AUROC were:

- LMT (77.1%, 94.2%, 94%, 77.9%, LR- (0.12), LR+ (4.1), 91.6 and AUROC 96)
- NBT (77.7%, 94%, 94.2%, 76.9%, LR- (0.08), LR+ (4), 90.6 and AUROC 91.9)
- ADT (84.4%, 94.3%, 92.4%, 77.9%, LR- (0.05), LR+ (4.4), 92.4 and AUROC 94.2)
- RF (88.5%, 93.5%, 97.5%, 74%, LR- (0.1), LR+ (3.6), 92.6 and AUROC 95.7)

Conclusion: Machine learning models can aid physicians in enhancing the early prediction of Non-viral Hepatocellular Carcinoma (NVHCC). Logistic Model Trees (LMT) sufficiently demonstrated the most significant performance at 0.96 and 92% for AUROC and Accuracy, respectively. Further validation will translate to cost-effective, personalized care of at-risk patients

PO7-05
Real-World Analysis of Systemic Therapy Sequences After Atezolizumab and Bevacizumab in Hepatocellular Carcinoma: Insights from the IMMUreal Cohort

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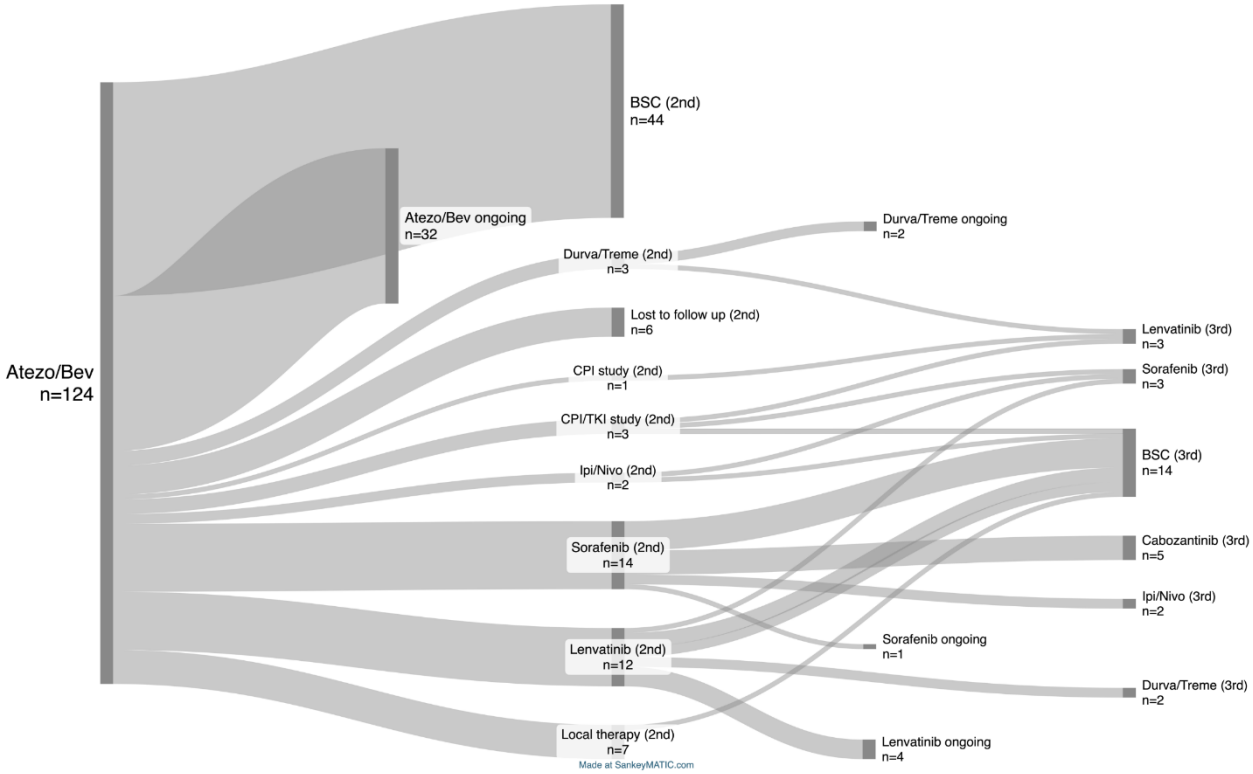
Background and aims: The introduction of several new systemic therapies in recent years has significantly altered the treatment landscape for advanced hepatocellular carcinoma (HCC). Despite these advances, the approval of atezolizumab/bevacizumab (atezo/bev) as the preferred first-line therapy over sorafenib has raised uncertainties regarding optimal treatment sequencing in advanced HCC. This study aims to assess sequential therapy for HCC following atezo/bev treatment and present evidence from a prospective real-world cohort.

Method: We report initial results from the ongoing IMMUreal cohort, which prospectively examines immunotherapies for HCC, recruiting patients from two tertiary centers in Bavaria. In this analysis, 124 patients treated with atezo/bev as first-line therapy between June 2020 and December 2023 were included. The structure and feasibility of sequential therapy were evaluated within defined prognostic subgroups.

Results: The median overall survival observed in this real-world cohort was 21.5 months. Only 41.2% of patients proceeded to second-line systemic therapy following atezo/bev, and the number of patients eligible for further treatment decreased, with only 19.2% receiving third-line therapy. This decline in eligibility for second- and third-line therapies was associated with a shorter duration of treatment between lines. Additionally, a significant deterioration in liver function, as indicated by ALBI and Child-Pugh scores, was observed after initiating systemic therapy. Notably, there was no correlation between the number of therapy lines received and adverse prognostic factors, such as liver cirrhosis, poor performance status, extrahepatic spread, or macrovascular invasion.

Conclusion: Sequential therapy following first-line treatment is feasible only for a subset of patients with advanced HCC, while liver function decline may limit the applicability of further treatments. New strategies for enhancing the effectiveness of multi-line therapies while preserving liver function is essential for improving outcomes in advanced HCC patients.


Atezo/Bev
n=124
BSC (2nd)
n=44
Atezo/Bev ongoing
n=32
Durva/Treme (2nd)
n=3
Lost to follow up (2nd)
n=6
CPI study (2nd)
n=1
CPI/TKI study (2nd)
n=3
Ipi/Nivo (2nd)
n=2
Sorafenib (2nd)
n=14
Lenvatinib (2nd)
n=12
Local therapy (2nd)
n=7
Durva/Treme ongoing
n=2
Lenvatinib (3rd)
n=3
Sorafenib (3rd)
n=3
BSC (3rd)
n=14
Cabozantinib (3rd)
n=5
Ipi/Nivo (3rd)
n=2
Sorafenib ongoing
n=1
Durva/Treme (3rd)
n=2
Lenvatinib ongoing
n=4
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PO7-06
Survival Outcome of Perioperative Radiotherapy in the Management of Cholangiocarcinoma

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Background and aims: Cholangiocarcinoma is a rare and aggressive form of cancer, accounting for approximately 3% of all gastrointestinal cancers. It arises from the biliary tract and often has a silent, progressive course. The main treatment for this condition is surgical resection of the tumor; however, the role of radiotherapy remains a topic of debate. The aim of the study was to assess the impact of the sequence of radiotherapy in relation to surgery on survival outcomes.

Method: Data was extracted from the Surveillance, Epidemiology, and End Results (SEER) database for patients diagnosed with primary cholangiocarcinoma between 2000 and 2020. All patients had undergone surgical management and were divided into two groups:those receiving preoperative radiotherapy and those receiving postoperative radiotherapy. SPSS version 23 was employed for data analysis, utilizing Kaplan-Meier curves and the Log-rank test for survival analysis

Results: We identified 1,075 patients with cholangiocarcinoma at various anatomical sites. The 3-year and 5-year age-standardized relative survival rates were 36.4% and 24.9%, respectively. For the preoperative radiotherapy group, the 3-year and 5-year relative survival rates were 40.4% and 33%, compared to 36.6% and 24.1% for the postoperative group (*P*=0.132).Approximately 16.65% of patients had localized disease, 56.74% had regional spread, and 15.53% had distant spread. Patients with localized disease had significantly higher survival rates compared to those with regional or distant spread 43.1%, 24.2%, and 7.6%, respectively; (*P*=0.0001). Regarding tumor grade, 40.7% of patients had Grade 1, 6.3% had Grade 2, 19.9% had Grade 3, and 0.65% had Grade 4 tumours. Grades 1 and 2 demonstrated better survival rates compared to Grades 3 and 4 28.2% and 31.7% cocompared to 17% and 15.2%.

Conclusion: Due to the rarity of cholangiocarcinoma, there are limited data available concerning the sequence of radiotherapy in relation to surgery. The results of this study suggest that postoperative radiotherapy offers very limited survival benefits when compared to preoperative radiotherapy. Additionally, localized stages showed a survival benefit of 36% compared to late stages, underscoring the importance of early detection and screening, particularly at the age of 65 to improve the quality of life and decrease further psychological and financial burden.

PO7-07
Living Donor Liver Transplantation for HCC patients with Macroscopic Portal Vein Tumor Thrombosis

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Background and aims: Macroscopic Portal vein tumor thrombosis (PVTT) was previously an absolute contraindication in liver transplantation (LT) for hepatocellular carcinoma (HCC). However, over time, some studies from high-volume Asian LDLT centers and subsequently from the western DDLT centers published good long-term survivals of PVTT patients by LT. The common characteristics of the patients in these studies were having low AFP (<100) and/or PIVKA-II and PVTT and PVTT classification between Vp1 and Vp3. In light of these data, we analysed the outcomes of LT in patients with PVTT at Liver Transplantation Institute.

Method: Data of LT patients with HCC on explant pathology were recorded retrospectively from the prospective and consecutively recorded HCC database of Liver Transplantation Institute of Inonu University, Malatya, Türkiye.

Results: Between 2006-2024, 602 LTs were performed for HCC in our institute. Of these 33 had Vp1 – Vp3 PVTT. We divided the Vp1-Vp3 patients (n=33) according to AFP cut off 100 ng/mL and analysed the overall survivals (OS). Vp1-Vp3 PVTT patients with AFP≤100 had 49.6% 5-year OS (Figure1). Then we divided the patients according to AFP (alpha-fetoprotein) and GGT (gamma glutamyl transferase) combinations. When we compared the survival of patients with low AFP (≤200) and low GGT (≤104) which is known as good prognostic markers from Expanded Malatya criteria, with other combinations of AFP and GGT. Vp1-Vp3 PVTT patients with AFP≤200 and GGT≤104 (n=10) had 100% 5-year OS (Figure2) and 68.6% 5-year disease-free survival (DFS) (Figure3).

Conclusion: In HCC patients with Vp1-Vp3 PVTT, if AFP≤200 and GGT≤104 were treated with liver transplantation, 5-year OS was 100% and 5-year DFS was 68.6%. These results are promising for patients with PVTT.

Figure 1. Overall survival graph of Vp1-Vp3 patients according to AFP cut off 100ng/ml

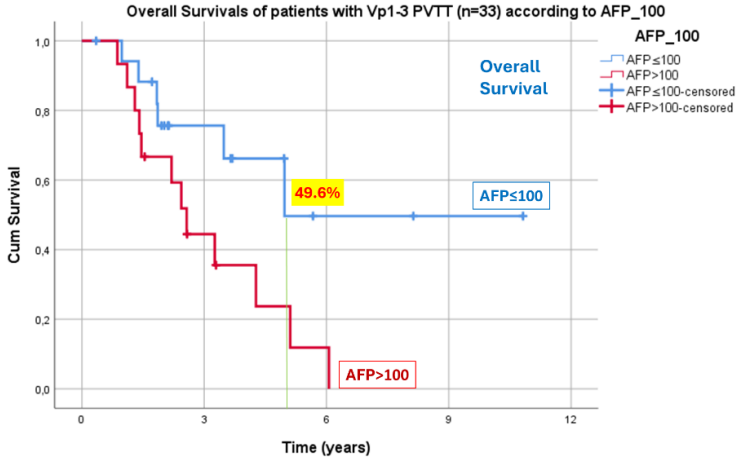


Figure 2. Overall survival graph of Vp1-Vp3 patients according to AFP and GGT combinations.

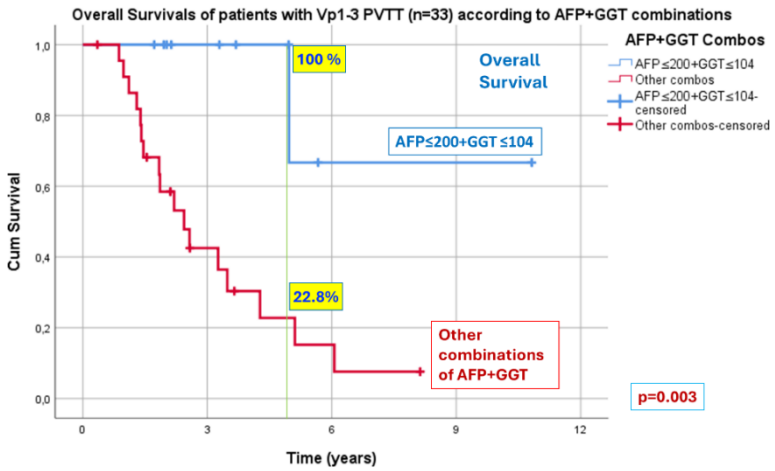
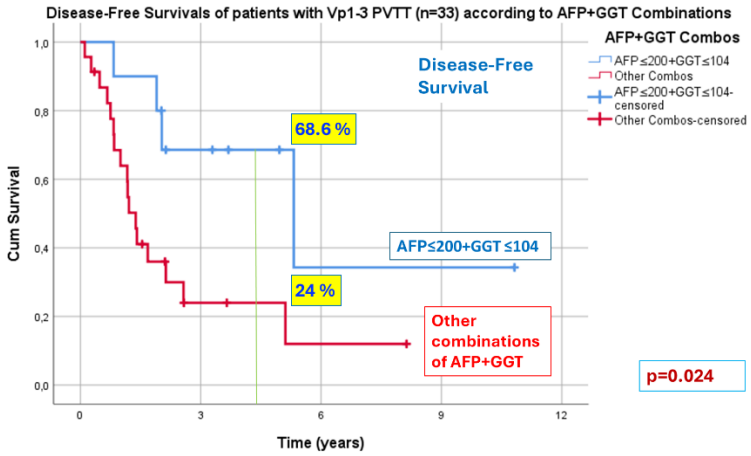


Figure 3. Disease-free survival graph of Vp1-Vp3 patients according to AFP and GGT combinations.



PO7-10
Stereotactic Radiotherapy as a Rescue Option for Patients with Hepatocellular Carcinoma Awaiting Liver Transplantation

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Background and aims: Several data indicate that stereotactic radiotherapy (SBRT) is an efficient strategy in patients with hepatocellular carcinoma (HCC) awaiting liver transplantation (LT). We used SBRT as a rescue option in patients considered as unsuitable for transarterial chemoembolization (TACE) or local ablation or after failure of one of this treatment. The objective of this study was to evaluate results of SBRT as a waiting treatment in patients enlisted for liver transplantation and considered as unsuitable for TACE or local ablation.

Method: All patients with HCC enlisted for LT who were treated by SBRT (45 Gy, 3 fractions) in a single center between 2013 and 2019 were included. Pathological response (PR) of treated nodules was reviewed by a pathologist.

Results: A total of 49 patients could be included. Median MELD score was 11 (range, 6 to 20) and 24 had already received at least one TACE. No hepatic decompensation was observed following SBRT. Of them, 39 could be transplanted after a median interval of 7 months (range, 1 to 39) since the end of SBRT with a nil post-transplant 90-mortality. A complete PR (CPR) was observed in 16 (43%) of the 37 nodules analyzed. Nodules with CPR were smaller than nodules with viable tumors (24 mm versus 34 mm; P=0.04). The time interval since the end of SBRT tend to be longer in the CPR group (9 versus 6 months; P=0.14). There was no difference in tumor location, MELD score or previous TACE. The 3-year disease-free survival after LT tend to be higher in the CPR group (83% versus 58%, P=0.058)

Conclusion: This series suggests the feasibility and the efficiency of SBRT in HCC patients awaiting LT and considered as unsuitable for TACE or local ablation, or after failure of one of this treatment.

PO7-14
Preliminary results from the hellenic registry for intrahepatic cholangio-carcinomas

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Background and aims: Intrahepaticcholangiocarcinomas(iCCs) represent an increasing type of CCs. In a 50% of ICCs there are genetic alterations amenable to target. The ESMO Scale for Clinical Actionability (ESCAT/ESMO) recommends molecular testing with next-generation-sequencing (NGS) in every iCC patient. We have created a data base to facilitate reimbursement of genetic testing in iCC-patients enrolled.

Method: After receiving a written informed consent, we haveprospectively registered dempographic, clinical and pathological data for patients with iCCs, the risk factors and the frequency of genetic alterations. A code for NGS analysis was requested.

Results: Duringtheperiod1/3/2023-9/2/2024, 80 patients were included (64 with complete genetic testing). A 81.5% ofpatientswere>60years(65.4% men), 1/3withBMI>25, 8.8% had cirrhosis and 15% had diabetes-MASLD. Five patients had HBV and two HCV infection. A 40.7% of patients presented extrahepatic spread on inclusion. The majority of patients with metastatic disease were commenced to first line therapy with cisplatin-gemcitabine-durvalumab(70%),achieving a disease control rate (DCR) of23.8%. The molecular testing depicted a genetic alteration in 45.3% of patients. In half of them a sigle mutation, in 20% two mutations and in another 20% genes rearegements. The most common mutation was detected in the genes *IDH1/2*(30%). Other existing molecular changes were mutations in NRAS (16%), KRAS (9.4%),PIKECA(9.4%),BRCA1(3.1%),ERBB2, as well as genes' fusion as FGFR2/3(6%) andC-MET(1.7%).Moreover, we detected combinations of genetic alterations asIDH2/PIK3CA, IDH1/BRCA1, IDH1/FANCA,IDH1/MET, KRAS/CKN2B,PIK3CA/BRCA1.

Conclusion: TheiCCregistryisactiveandtheinformationretrievedwill offer a better understanding of this cancer and facilitate a more targeted and affective management.

PO7-15-YI
The tumor burden score is a novel prognostic indicator for intermediate-stage hepatocellular carcinoma patients receiving transarterial chemoembolization: A preliminary single-center study

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Background and aims: Tumor burden is a significant determinant of hepatocellular carcinoma (HCC) prognosis. Recent research has demonstrated that the tumor burden score (TBS) promotes tumor involvement; however, the prognostic accuracy of various treatment strategies remains to be established. This single-center study aimed to evaluate the prognostic efficacy of TBS in patients undergoing transarterial chemoembolization (TACE).

Method: A cohort of 178 treatment-naive HCC patients receiving TACE was enrolled in this study. We evaluated the impact of TBS on the treatment response, development of vascular invasion, and extrahepatic spread after TACE. Univariate and multivariate logistic regression analyses were conducted to identify the independent factors associated with mortality.

Results: Among the 178 patients, the majority presented with a single tumor (n = 114, 64 %) with a median size of 4.0 cm (IQR, 2.8–6.0), and BCLC stage B (n = 92, 51.7 %). The median TBS was 4.1 (IQR, 3.2–6.1), and the median survival was 28 months (IQR: 18-58). The TBS was significantly correlated with vascular invasion (p = 0.03); however, no significant association was observed with metastasis (p = 0.38). Elevated TBS was associated with stable disease (p = 0.02, OR: 1.381, 95 % CI: 1.122-1.661) and progressive disease (p = 0.01, OR: 1.381, 95 % CI: 1.151-1.658) following TACE. In multivariate analysis, sex, age, etiology of liver disease, MELD-Na, ALBI Score, alpha-fetoprotein, and BCLC stage were not significantly associated with survival. TBS emerged as an independent risk factor for mortality after adjusting for other factors in the multivariate analysis (p = 0.03, HR: 1.368, 95 % CI: 1.03-1.815).

Conclusion: TBS serves as a promising indicator of treatment response following TACE. An elevated TBS is indicative of vascular invasion, disease progression, and mortality in HCC.

PO7-17
Analysis of disease-specific therapeutic efficacy of atezolizumab plus bevacizumab in advanced hepatocellular carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) presents diverse clinical features affected by tumor size, lesion count, metastasis, and vascular invasion, contributing to complex disease pathology. The combination of atezolizumab plus bevacizumab (Atez/Bev), which synergistically combines anti-PD-L1 and anti-VEGF antibodies, has emerged as the first-line treatment for advanced HCC. This study analyzes Atez/Bev efficacy in advanced HCC patients, examining therapeutic responsiveness across diverse disease profiles.

Method: We conducted analyses of both overall patient outcomes and individual nodule responses to Atez/Bev in advanced HCC patients at our institution. Patient assessment included key clinical factors such as extrahepatic metastasis (EHM), major vascular invasion (MVI), high AFP (≥ 400ng/mL), and large intrahepatic tumors (≥ 5cm). Notably, our analysis of individual nodules tracked temporal changes in tumor dynamics by monitoring up to five intrahepatic nodules per case, allowing precise evaluation of tumor-specific responses through longitudinal diameter measurements. For each clinical factor (EHM, MVI, elevated AFP and large intrahepatic tumors), we evaluated treatment efficacy using the following endpoints: target lesion reduction rate, intrahepatic nodule time-to-progression (TTP), progression-free survival (PFS) and overall survival (OS).

Results: Among 183 cases (517 nodules), MVI presence had no significant effect on target lesion reduction ($p = 0.556$), and no complete responses occurred with large intrahepatic tumors. While EHM, MVI, and elevated AFP did not significantly impact intrahepatic nodule TTP, large tumors showed prolonged TTP compared to those with smaller tumors ($p = 0.08$). Multivariate analysis showed PFS was significantly associated with EHM ($p = 0.02$), while OS was associated with EHM ($p < 0.01$), elevated AFP ($p = 0.03$), and large intrahepatic tumors ($p < 0.01$). Progression patterns showed equal rates of lesion enlargement and new lesions in cases with large tumors, with a lower rate of PD due to lesion enlargement compared to other cases. A correlation between PFS and OS was noted in large intrahepatic tumors ($r = 0.659$ for ≥ 5cm, $r = 0.483$ for < 5cm).

Conclusion: In this analysis of Atez/Bev efficacy across diverse HCC profiles, therapeutic responses varied depending on disease characteristics. While the treatment maintained efficacy in cases with MVI, the presence of large intrahepatic tumors significantly influenced outcomes, with a notably stronger PFS-OS correlation in large tumor cases. These findings suggest the importance of considering intrahepatic tumor size as a stratification factor in future clinical trials of systemic therapy for advanced HCC.

PO7-18-YI
The Size - Growth Rate Relationship in Hepatocellular Carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related mortality globally, resulting in approximately 800,000 deaths annually. Regular surveillance has been shown to improve survival rates. Currently, the European Association for the Study of the Liver (EASL) recommends a six-month surveillance interval based on historical tumour growth kinetics. However, this guidance assumes a relatively consistent growth rate, which may vary with initial tumour size. This study aims to explore the relationship between HCC initial size and growth rate, hypothesizing that larger tumours may grow more slowly due to biological constraints affecting cellular replication.

Method: A prospective study was conducted at a single tertiary centre with approval from the Nottingham University Hospital Clinical Effectiveness Board (19-223C). We included 144 HCC lesions from 65 patients who underwent at least two cross-sectional imaging studies of same modality (CT or MRI) before treatment. HCC lesions were confirmed based on Liver Imaging Reporting and Data System (LI-RADS) or histologically. Growth rates were calculated by measuring lesion diameters on initial and follow-up imaging and expressing the size change per month. Two hepatobiliary radiologists independently assessed the images, achieving high interobserver reliability ($R^2 = 0.8339$). Growth rate correlations with initial tumour size were analysed.

Results: Our analysis revealed a statistically significant correlation between initial tumour size and growth rate per month (Spearman Rho = 0.526, $p < 0.001$). Grouping tumours by an initial size cutoff of <100 mm and >100 mm, those with an initial size >100 mm exhibited a notably higher median growth rate (3.9 mm/month [IQR 1.8–13.5]) compared to smaller tumours (1.4 mm/month [IQR 0.5–3.1], $p = 0.019$). Additionally, growth patterns indicated an increase in rate up to an initial size of 100 mm, plateauing and eventually declining beyond 120 mm.

Conclusion: HCC growth appears to follow a logarithmic model, with rapid early growth that decelerates as tumour size increases, potentially due to biological factors like mutational burden or vascular limitations. Further research is warranted to determine optimal surveillance intervals for varying HCC sizes and to inform tailored treatment approaches based on tumour biology and growth patterns.

PO7-19
Hepatic arterial infusion chemotherapy combined with camrelizumab and lenvatinib in the treatment of advanced hepatocellular carcinoma: a retrospective study

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Background and aims: Programmed cell death protein-1 (PD-1) inhibitors, in combination with tyrosine kinase inhibitors (TKIs), have achieved significant therapeutic effects in unresectable hepatocellular carcinoma (HCC).
Hepatic arterial infusion chemotherapy (HAIC) based on the FOLFOX regimen has also shown promising response rates and survival benefits for advanced HCC. This study aimed to retrospectively analyze the efficacy and safety of HAIC combined with camrelizumab (a PD-1 inhibitor) and lenvatinib in the treatment of advanced HCC.

Method: This study included patients diagnosed with HCC who received at least three cycles of HAIC combined with camrelizumab and lenvatinib at Harbin Medical University Cancer Hospital from November 2020 to February 2023. We evaluated patient characteristics, treatment regimens, tumor responses, survival outcomes and adverse events (AEs).

Results: A total of 64 patients received HAIC combined with lenvatinib and camrelizumab. Of these, the tumors of 21 patients were transformed into lesions that could be surgically resected including16 patients underwent surgical resection, 1 patient underwent radiofrequency ablation with satisfactory recovery, and 4 opted to forgo surgery. According to the mRECIST criteria, 16 patients achieved complete response, 33 patients achieved partial response, 7 patients had stable disease, and 8 patients had progressive disease, resulting in an overall response rate (ORR) of 76.6%. When assessed by RECIST v1.1, the ORR was 54.7%. Tumor response with different clinical stages was evaluated by RECIST v1.1 and revealed the best ORR in stages IB and IIIA. The median follow-up time was 17.9 months, and the median progression-free survival (PFS) was 15.73 months. Survival analysis indicated that PFS was significantly longer for Child-Pugh A patients compared to those with Child-Pugh B ($P < 0.05$), while no significant differences were noted in other subgroup analyses. All AEs were deemed tolerable and manageable, with no treatment-related deaths reported during follow-up.

Conclusion: This study demonstrated that for patients with advanced HCC, the combined use of HAIC, lenvatinib and camrelizumab showed controllable safety and good efficacy. The tumor lesions of most patients were significantly relieved, with 32.8% of the patients were converted to a resectable state, reflecting the obvious tumor shrinkage effect of the triple regimen.

PO7-20-YI
EXTRAHEPATIC CHOLANGIOCARCINOMA LANDSCAPE IN ROMANIA

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Background and aims: Perihilar cholangiocarcinoma is a diagnosis which still poses a challenge for health care systems. As most patients are often diagnosed in unresectable stages, palliative biliary drainage and systemic treatment become the therapeutic targets; however it can often prove difficult to obtain a histological diagnosis and to achieve efficient biliary drainage in these patients.

Method: We retrospectively evaluated 276 patients with perihilar cholangiocarcinoma treated in our center between January 2018-April 2024. We reviewed patients performance status (ECOG), bilirubin levels, whether or not a histological diagnosis was obtained and through which method, biliary drainage method chosen and whether it was successful (i.e. decrease in bilirubin levels with at least 50%), 30-day mortality, complications.

Results: We included a total of 276 patients with a mean age of 68 years, most patients being diagnosed with Bismuth III & IV tumours. Only 8.5% of patients had resectable tumours. Only 55.11 % of patients had a histological diagnosis. Most biopsies were obtained during endoscopic drainage (fluoroscopic guidance or cholangioscopy), with a diagnostic accuracy of 76%, followed by endoscopic ultrasound guided fine needle aspiration with an accuracy of 83%; cell block cytology from bile obtained during endoscopy, less challenging and less time consuming, has proven to be a useful tool, with a similar diagnostic accuracy (74%). Endoscopic drainage was the preferred method of biliary drainage, with a success rate of 53.33%; however when patients underwent percutaneous drainage per primam the procedure had a 80% success rate, a significant difference (p=0.018). The majority of patients for which endoscopic drainage wasn't successful were classified as Bismuth IV, in this category percutaneous drainage being more effective (78% vs 45%, p=0.02). In patients with Bismuth III and IV and a poor ECOG score endoscopic drainage has a markedly lower success rate (27.58%), in-hospital mortality being high even when drainage is efficient (62.5% in-hospital mortality), mainly due to post-procedural complications such as cholangitis.

Conclusion: Hilar cholangiocarcinoma carries a poor prognosis, with under 10% of patients being diagnosed in resectable stages. A significant number of patients still do not obtain a histological diagnosis; bile cell block cytology could prove useful in improving diagnostic accuracy in these patients. In Bismuth IV tumours percutaneous drainage is more efficient than the endoscopic one. In a subcategory of patients, Bismuth III-IV with poor performance status (ECOG 3-4), with poor short-term prognosis, endoscopic drainage carries high mortality rates and high rates of periprocedural complications.

PO7-21
Predicting Hepatocellular Carcinoma in CHC patients using FIB4 and FIB6: A Longitudinal Study on non-invasive risk assessment models

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Background and aims: Hepatocellular carcinoma (HCC) remains a leading cause of mortality among hepatitis C (HCV) patients, even after achieving a sustained virological response (SVR) through direct-acting antivirals (DAAs). Non-invasive fibrosis scores have emerged as valuable tools for stratifying HCC risk among these patients, potentially enabling more efficient, less invasive follow-up strategies. Our aim is to evaluate the predictive performance of non-invasive fibrosis scores FIB-6 versus FIB-4 for the prediction of de novo HCC development in CHC patients who achieved SVR

Method: We conducted a prospective study including CHC patients with advanced chronic liver disease (ACLD) who achieved SVR were enrolled at the Egyptian liver research institute and hospital (ELRIAH) from Between January 2020 and December 2022. These patients were analayzed for the following parameters of liver fibrosis: Fibrosis 4 score (FIB-4) and FIB-6 . The diagnostic accuracy of tests was evaluated by Harrell's c-statistic , Brier score and Hosmer–Lemeshow test p-value

Results: In this study of 492 patients, 21.1% were at fibrosis stage F3 and 78.9% at stage F4. The mean follow-up duration was 68.54 ± 22.97 months, during which 35 cases of HCC developed, with an incidence rate of 1.246/100 person-years. For FIB4 (cutoffs: 1.45 and 3.25), HCC incidence was 0.25%, 1.4%, and 1.45% in low-, intermediate-, and high-risk groups, respectively, with a low Harrell's c-statistic (0.128) Brier score was 0.791 and Hosmer–Lemeshow test p-value was 0.088. Using FIB6 (cutoffs: 2.48 and 2.62), HCC incidence was 0.13%, 1.06%, and 1.75% in corresponding risk groups, showing significant incidence increase with higher scores (p=0.033) and a fair Harrell's c-statistic (0.666), Brier score was 0.667 and Hosmer–Lemeshow test p-value was 0.322.

Conclusion: FIB6 outperformed FIB4 in prediction of HCC in CHC patients who cleared HCV. FIB6 demonstrated a better correlation with HCC development, especially in identifying high-risk patients. The significant association between FIB6 scores and HCC incidence supports its potential utility for long-term monitoring in clinical settings, enhancing early detection strategies for high-risk populations. Future studies should explore integrating these non-invasive markers with other clinical factors to refine HCC prediction models further.

PO8-01
Insights into the GALAD Score: a new optimal cut-off for hepatocellular carcinoma

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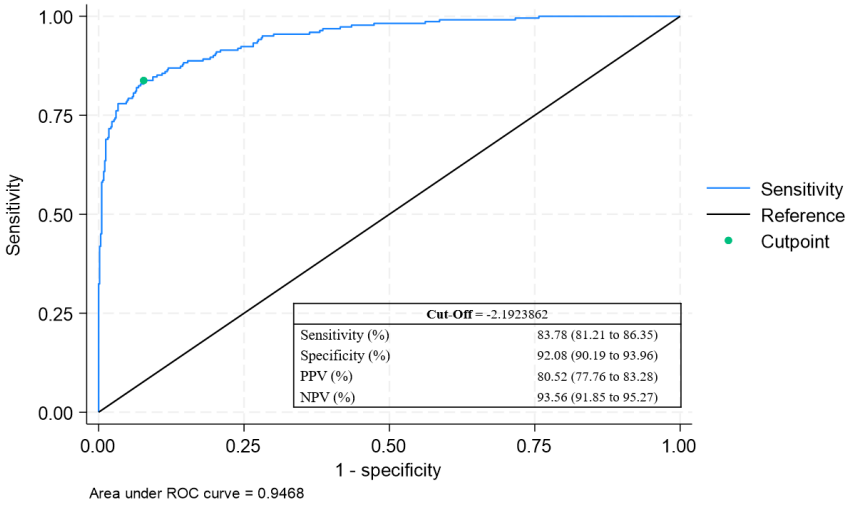
Background and aims: Hepatocellular carcinoma (HCC) affects 70-95% of people with chronic liver disease (CLD). Men aged 40 to 60 in Western countries, are more likely to develop HCC. Abdominal ultrasonography (US) is recommended as a semiannual screening method for patients at higher risk of HCC by various international guidelines, whereas serum alpha-fetoprotein (AFP) is inconsistently recommended. HCC surveillance could be improved by combining AFP, AFP-L3, and des-gamma-carboxy prothrombin (DCP). These biomarkers, along with age and gender, were combined into an algorithm to create a GALAD score, which has a higher overall accuracy for early detection of HCC than the sum of its component biomarkers. However, no studies have established a standardized GALAD cut-off due to a lack of biomarker value comparisons between healthy people and those with cirrhosis or HCC.

Method: In this cross-sectional and multicenter Italian study with a prospective design, we examined four cohorts (n = 1324), and divided them into steadily (at two different long-term times) healthy individuals (n = 568), cirrhosis of any aetiology (n = 534), and HCC (n = 222). These participants were drawn from an Italian historical cohort that included patients from the IRCCS "S. de Bellis" Gastroenterology Department, the University of Modena and Reggio Emilia Gastroenterology Department, and Padua University Hospital.

Results: Healthy subjects were significantly younger (53.51 ± 13.73 years) than those with cirrhosis or HCC (60.53 ± 12.14 and 66.69 ± 10.16 years, respectively). GALAD scores differed significantly between groups, with the healthy group having a lower level of -4.37 ± 1.67 compared to the values of the cirrhosis and HCC groups (-1.98 ± 2.35 and 0.74 ± 3.41, respectively). A GALAD score cut-off of -2.19 was determined, with sensitivity and specificity of 83.78% and 92.08%, respectively. Individuals with GALAD values > -2.19 have a moderate-to-very high risk (approximately 90%) for developing HCC.

Conclusion: GALAD was the most effective score for discriminating HCC among patients with compensated advanced CLD without HCC at a new optimal cut-off implemented for the first time in a large and "steadily healthy" population. We strongly recommend incorporating this cut-off into clinical national/international guidelines for high-risk patients undergoing HCC screening and surveillance.

Figure 1: Area under curve (ROC) of GALAD score and optimal cut-off, with confidential interval at 95%.



PO8-02-YI

Early changes in body composition parameters following locoregional treatment are associated with survival and need for liver transplantation in cirrhotic patients with hepatocellular carcinoma

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Background and aims: Radiofrequency thermo-ablation (RFTA) and trans-arterial chemoembolization (TACE) are known to be effective treatment strategies for cirrhotic patients with hepatocellular carcinoma (HCC). Body composition parameters, especially skeletal muscle index (SMI), have been suggested to predict outcomes in HCC patients. However, few studies have examined the impact of visceral and subcutaneous adipose tissue indices (VATI and SATI) on patient outcomes, and no data are available on the effects of their post-treatment changes.
Therefore, this study aims to assess the impact of early post-treatment changes in body composition parameters on the overall-survival (OS) of cirrhotic patients with HCC and on the probability of being transplanted.

Method: Consecutive cirrhotic patients with HCC who underwent their first locoregional treatment with TACE or RFTA from 2012 to 2022 were retrospectively enrolled. Body composition parameters (SMI, SATI and VATI) were extrapolated from abdominal CT scan performed before and one month after treatment using Slice-O-Matic software. Changes in these parameters were expressed as DELTA, calculated using the formula: (one-month value - pre-procedure value)/ elapsed time in days. Overall survival (OS) and probability of LT were assessed using Fine-Gray multivariate competing risk analysis, considering, respectively, LT and HCC progression beyond the 'up-to-seven' LT criteria as competing risk events.

Results: 189 patients were enrolled, 132 undergoing TACE and 57 RFTA. In the multivariate analysis, across the entire population, the early decrease of VATI (DELTA-VATI negative) one month after treatment was associated with an increased risk of death (SHR=1.636; 95.0% CI=1.230-1.979; P=0.018), adjusting for age, MELDNa, Up-to-seven status and DELTA-SMI. In a sub-analysis, conducted considering only patients potentially eligible for LT, the early decrease in SMI (DELTA-SMI negative), but not in VATI was significantly associated to the need for LT (SHR=5.155; 95.0% CI=4.212-6.098; P<0.0001).

C**onclusion:** early body composition parameters post-treatment changes are useful in identifying cirrhotic patients with HCC at increased risk of death or need of LT. Further studies on larger cohorts are needed to confirm these findings.

PO8-03-YI
Assessment of Immunotherapy alone (Atezolizumab/Bevacizumab) or combined with trans-arterial chemoembolization for treatment of intermediate stage-B3 Hepatocellular Carcinoma

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Background and aims: Trans-arterial chemoembolization (TACE) is the most frequently used initial treatment for the Barcelona clinic of liver cancer (BCLC) intermediate stage-B hepatocellular carcinoma (HCC). Systemic therapy is proposed for BCLC-B3 with extensive hepatic involvement. We aimed to compare treatment response and overall survival (OS) in patients with intermediate stage-B3 HCC treated with combined Atezolizumab/Bevacizumab (A/B) and TACE versus those treated with Atezolizumab/Bevacizumab alone

Method: This retrospective study recruited patients with intermediate stage-B3 HCC who received combined Atezolizumab/Bevacizumab (A/B) and TACE (group-1) versus those treated with Atezolizumab/Bevacizumab alone (group-2). We compared the demographic, laboratory and tumor characteristics and response of the studied groups. OS was calculated from the date of treatment start till patients' death or the study end in October 2024.

Results: This study included 20 patients treated with combined Atezolizumab/Bevacizumab (A/B) and TACE (group-1) and 17 patients treated with Atezolizumab/Bevacizumab alone (group-2). There were no significant differences between the groups in the median age (65.50 vs 60.00 years respectively, p 0.125), male predominance (60.0% vs 64.7%, p 0.769), underlying aetiology (hepatitis C/B/non-viral: (95.0%/0.0%/5.00% vs 76.5%/5.9%/17.60%, p 0.459) , median alph-fetoprotein (26.65 vs 180.00,p 0.619), albumin/bilirubin (ALBI) score (-2.83 vs -2.39, p 0.133), or adverse events (AEs) (75.0% vs 47.1%, p 0.081).
All included patients had Child-Pugh A and BCLC-B3. We found significant differences between the groups in the median platelet count (143.00 vs 263.00, p 0.002), Diabetes mellitus (55.0% vs 17.6%, p 0.020), and HCC size (6.95 vs 9.50 cm, p 0.033).
The median (IQR) number of TACE sessions in group-1 was 2.00 (2.00 - 3.00). The median number of immunotherapy cycles was (12.00 vs 9.5 cycles, respectively, p 0.774). Tumor response by the RECIST criteria was (partial response/stable disease/progression: 25.0%/65.0%/10.0% vs 11.8%/82.4%/5.9%, p 0.552). The prevalent AEs in group-1 were hypertension (15%), abdominal pain (10%), Anorexia (5%), fatigue (5%), Thyroditis (5%) and skin rash (5%). The prevalent AEs in group-2 were hypertension (11.8%), diarrhea (11.8%), abdominal pain (5.9%), Autoimmune Thyroditis (5.9%), epistaxis (5.9%).
By the end of the study, 2 patients died in group-1 (10.0%) and 2 patients died in group-2 (11.8%). The median (IQR) OS was significantly longer in group-1 compared to group-2 (13.27 (11.87 – 16.08) vs 9.10 (6.97 – 12.20) months, respectively, p 0.007)

Conclusion: OS was significantly longer in patients who received combined immunotherapy and TACE compared to patients who received immunotherapy alone with no difference in tumor response or AE

PO8-04
HCC is the leading indication for liver transplantation in HCV-infected patients in the direct-acting antivirals era (single centre experience)

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Background and aims: The estimated prevalence of HCV in Czechia was 0.62% in 2015. Despite the low prevalence, HCV infection was the second most frequent indication for liver transplantation (LTx) before 2014. The introduction of direct-acting antivirals (DAAs) in the treatment of chronic HCV infection in April 2014 resulted in a rapid decline in liver transplants for HCV. Our study aimed to describe the changing landscape of LTx for HCV in the DAAs era**.**

Method: We retrospectively evaluated the clinical records of 239 patients who underwent LTx owing to HCV between 1995–2024. 152 patients were transplanted before 2014 in the interferon-based (IFN) treatment era and 87 in the DAA treatment era. The study included patients indicated to LTx for chronic liver failure or hepatocellular carcinoma (HCC).

Results: LTx for HCV was more frequent in the IFN era (152/1080, 14.0% recipients) in comparison with DAA era (87/1464, 5.9% recipients, p< 0.0001). The patients in the DAA era were older (median age 53.6, vs 61.3 years, p < 0.0001). DAA cohort included a significantly higher proportion of individuals with HCC (51/152, 33.9% vs 62/87, 71.3%, p < 0,0001). Sustained virological response (SVR) before LTx was achieved in only two patients transplanted in the IFN era, and 42 patients of the 117 who were treated after LTx achieved an SVR (SVR rate 35.9%), 33 patients were contraindicated to the IFN treatment. All patients treated with DAA achieved an SVR (100%), 31/87 before LTx and 55 after LTx. The 5-year survival of patients transplanted in the DAA era was higher than that of those transplanted in the IFN era (79 vs 71%, respectively); the 10-year survival did not differ between groups (63 vs 59%, respectively). Positive predictive factors of 5-year survival were anti-HCV treatment administration and SVR achievement (OR 3.6, 95% CI 1.2–11.1 and 9.1, 95% CI 3.2–28.4, respectively). Fibrosing cholestatic hepatitis after LTx was a negative predictive factor of 5-year survival (OR 0.2, 95% CI 0.08–0.67). HCC as an indication for LTx, LTx in the IFN era, sex, and age at LTx did not affect the 5-year survival.

Conclusion: The risk of HCC persists despite the successful DAA treatment of chronic HCV infection in cirrhotic patients. LTx is still a suitable treatment option for patients with HCV-associated HCC. Our data support the need for careful HCC surveillance in all HCV patients achieving an SVR in the DAA era.

PO8-05-YI
Hepatocyte Nuclear Factor 1 Alpha Variants as Risk Factor for Hepatocellular Carcinoma Development with and without Diabetes Mellitus

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Background and aims: hepatocyte Nuclear Factor 1 Alpha (HNF1A) gene variants have been reported to be involved in developing mature onset diabetes mellitus (DM). Many studies reported the role of DM as a risk factor for hepatocellular carcinoma (HCC) development. To date, it has not been reported whether HNF1A gene variants are associated with the risk of DM in cirrhotic patients and their subsequent HCC. We evaluated the HNF1A genetic variants as a cofactor with DM for HCC development in hepatitis C virus (HCV)-infected patients.

Method: this study was conducted on 140 subjects; 30 had HCC without DM, 30 HCC with DM, and 40 patients had DM with no HCV infection or had HCC; in addition, 80 healthy volunteers with matched ages and genders were enrolled in the study as a control group. Liver function tests, hepatitis viral markers, alpha-fetoprotein (AFP), fasting sugar and HBA1c and HNF1A (rs2464196 and rs1169310) using real-time polymerase chain reaction (PCR) were done for all participants.

Results: the frequency of HNF1A rs2464196 (AA) genotype in patient groups (DM, HCC, HCC+DM) was significantly higher compared to the control group (P=0.006, P=0.018, P<0.001 respectively). The combined dominant model (AA + GA) of rs2464196 was significantly higher than the (GG) genotype in patient groups (DM, HCC, HCC+DM) than the control group. In addition, the frequency of the AA genotype is more prevalent in HCC+DM (73%) compared to the group of DM or HCC patients. In contrast, the HNF1A rs 1169310 (TT, TC or CC genotypes) showed no significant difference among the four studied groups and their T or C allele distributions.

Conclusion: This finding suggested that the HNF1A rs2464196 (AA) genotype could be associated with DM and may raise the possibility of HCC development among HCV-infected patients who harbour this genotype more than (GG). On the contrary, the HNF1A rs1169310 polymorphism was of no significance as a risk factor in the current study. Large scale studies are recommended on other variants of HNF1A to clarify the role of this gene in HCC development.

PO8-07
M2BPGi associated with the risk of hepatocellular carcinoma in patients with chronic hepatitis

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Background and aims: The Mac-2 binding protein glycosylation isomer (M2BPGi) in serum is a biomarker for liver fibrosis that has been widely studied in chronic liver diseases. This study examines the role of M2BPGi, a novel serum marker, in predicting hepatocellular carrcinoma (HCC) in patients with chronic hepatitis B (CHB)

Method: A cross-sectional descriptive study design. The study subjects included patients at the Hepatitis Clinic of University Medical Center Ho Chi Minh City from January 2023 to July 2024. M2BPGi quantification was performed using an immunoassay with a commercially available kit (HISCL M2BPGi; Sysmex Co., Kobe, Japan) and a fully automatic immunoanalyzer (HISCL-5000; Sysmex Co.)

Results: 165 HCC (125 males and 40 females) had a median age of 61.0 years (range, 32 to 88); 103 chronic hepatitis patients (64 males and 39 females) had a median age of 44.0 years (range, 22 to 85) have a level of liver fibrosis below stage F2 according to the Metavir classification as control group. *Serum M2BPGi Level and HCC Development*: For the whole HCC group (n = 165); a cutoff value of M2BPGi level ≥ 1.32 cut off index (COI) yielded an AUROC of 0.762; Se of 69.7%; Sp of 75.7%; PPV of 81.7%; NPV of 61.1%; the OR of 7.01 (95% confidence interval, 4.02-12.22) indicates that patients with M2BPGi high levels more likely to have HCC compared to those with lower levels. For HCC non-cirrhotic group (n = 99), a cutoff value of M2BPGi level ≥ 1.22 cut off index (COI) yielded an AUROC of 0.794 (p = 0.03); Se of 79.8%; Sp of 73.8%; PPV of 74.5%; the NPV of 79.2%; the OR of 11.11 (95% confidence interval, 5.75-21.47). These results suggest that M2BPGi is a useful tool in detecting HCC in CHB patients without cirrhosis. *M2BPGi compared to AFP:* the AUROC of M2BPGi had a significantly higher AUROC value than AFP among patients with HCC (0.762 versus 0.612; p = 0.000). In the HCC non-cirrhotic patient group, M2BPGi also shows a significantly higher AUROC than AFP (0.794 versus 0.695; p = 0.03). In the cirrhotic HCC patient group (n = 66), M2BPGi again demonstrated a significantly higher AUROC compared to AFP (0.715 versus 0.487; p = 0.04).

Conclusion: The incidence of hepatocellular carcinoma (HCC) is significantly associated with M2BPGi levels. M2BPGi can be used as a useful tool to detect hepatocellular carcinoma in patients with chronic

PO8-08
Lenvatinib is highly effective in patients with hepatocellular carcinoma related to both non alcohol-related steatohepatitis and alcohol-related etiology: a propensity score analysis

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Background and aims: Incidence of hepatocellular carcinoma (HCC) in non-alcoholic steatohepatitis (NASH)-related liver cirrhosis is on the rise. Therefore, it is crucial to identify effective treatments in these patients. Aim of this study was to investigate the effectiveness and safety of lenvatinib in this setting, compared to patients with alcohol-related etiology.

Method: Data on a multicenter series of 378 HCC patients treated with lenvatinib between 2019 and 2024 were analyzed. Propensity score matching was performed based on several covariates including age, sex, tumoral stage, alpha-fetoprotein (AFP) levels and Child-Pugh score. Survival estimates were computed by means of Kaplan-Meier method and compared with log-rank test. Results were expressed in terms of hazard ratio (HR) and 95% confidence interval (CI).

Results: After matching, 115 patients in each group were compared. Median overall survival was 21 months (95% CI: 20-23) in the group with NASH and 19 months (18-21) in the group with alcohol etiology (p=0.18). In multivariate analysis, only Child-Pugh stage (HR 2.67, 1.84-5.41), and tumoral stage (HR 2.18, 1.57-6.93) resulted as significant predictors of overall survival. Median progression-free survival was 9 months (8-9) in patients with NASH and 9 months (7-10) in patients with alcohol etiology (p=0.33). Only Child-Pugh score resulted as a significant predictor of progression-free survival in univariate analysis (HR 1.56, 1.15-3.41; p=0.03). No difference in terms of adverse event rate was observed between the two groups.

Conclusion: Lenvatinib is highly effective in patients with both NASH-related HCC and with alcohol-related etiology.

PO8-10
Setting the record straight: Utility and outcomes of HCV treatment in patients with HCV related HCC

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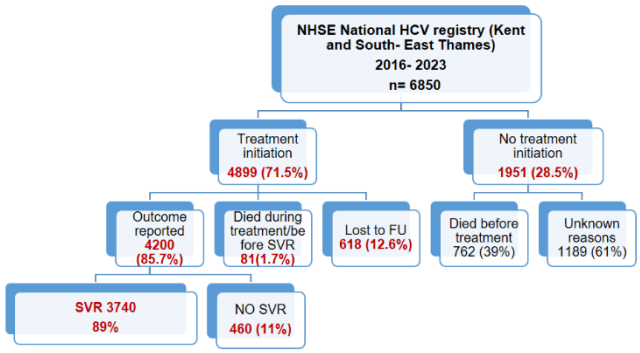
Background and aims: Achieving a sustained virological response (SVR) after hepatitis C virus (HCV) treatment leads to an improvement in patient survival rates across the whole spectrum of the disease, including those patients with HCC. However, direct acting antiviral (DAA) therapy has not been broadly recommended in clinical practice to those patients with HCC. NHS England approved DAA therapy for all viraemic patients, including those with HCC. The aim of this retrospective study is to provide a real-life data analysis of HCV treatment in those with HCC since the introduction of DAAs.

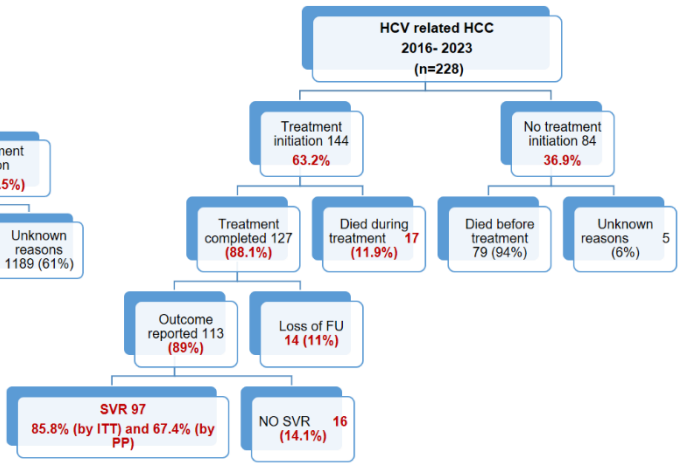
Method: Patients with HCV related HCC from the National Hepatitis C registry in South-East England between 2016 and 2023 were retrospectively included. The primary outcome was to assess the HCV care cascade (treatment initiation, outcome reported and SVR status) in patients with HCV related HCC (HCC cohort) in comparison with those without cancer (non- HCC cohort).

Results: 228/6850 (3.3%) HCV related HCC patients were identified. 71.8% (4755/6622) from the non-HCC cohort and 63.2% (144/228) from the HCC cohort initiated DAA therapy ($p<0.05$). By per protocol analysis, SVR was 76.6% (3643/4755) and 67.4% (97/144) in the non-HCC cohort and HCC cohort respectively ($p= 0.010$). By ITT, SVR rate in HCC cohort was 85.8% (97/113) vs 89.3% (3643/4081) in the non-HCC cohort ($p =0.992$). 11% (17) in the HCC cohort and 3.5% (168) in the non-HCC cohort were re-treated ($p<0.05$).
Patients in the HCC cohort were older (67 vs 55 years; $p<0.05$), had a significant greater degree of fibrosis (moderate fibrosis, 22.6 vs 7.1%, cirrhosis 55.6 vs 18.7%; $p<0.05$), were infected with genotype 3 (41 vs 27.3%, $p<0.05$), had higher alcohol intake (31 vs 15%, $p<0.05$) and required sequential treatment with more than one DAA regimen (11.8 vs 3.5%, $p<0.05$) in comparison with those without HCC
At the time of DAA therapy 88% had an active HCC and the majority (64%) had early stage cancer. SVR rate was 95%, 56.3% and 78% in BCLC 0/A, B, and C respectively. The median overall survival after DAA therapy was 105, 32, 18 and 4 months for BCLC 0-A/B/C/D respectively.

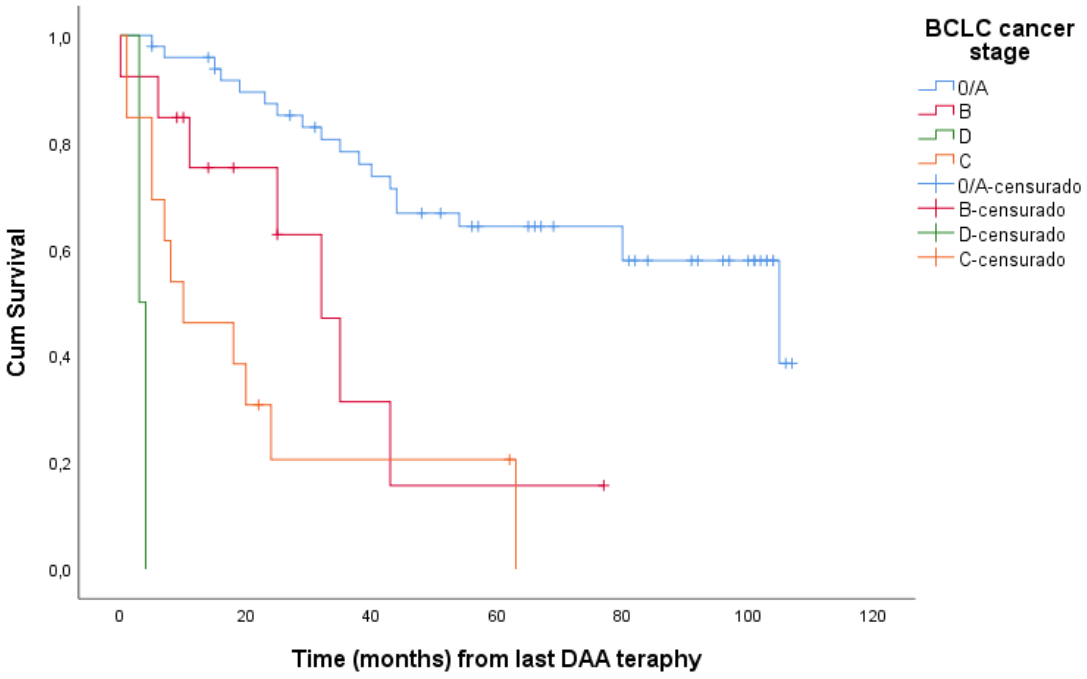
Conclusion: More than two thirds of patients with HCV-related HCC initiated and completed DAA therapies in South-East England. This high level of treatment uptake has led to an acceptable cure rate. Treating patients with HCV and HCC should be viewed as an appropriate clinical standard to improve overall outcome in patient with HCC.



Flow chart of Hepatitis C care by per protocol (PP) and intention to treat (ITT).



Flow chart of Hepatitis C care cascade in patients with HCC by PP and ITT.



PO8-11
Real-world efficacy and safety of first-line Gemcitabine-Cisplatin-Immune Checkpoint Inhibitor in patients with advanced biliary tract cancers: a single-centre experience

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Background and aims: Biliary tract cancers (BTC) are a group of rare but deadly cancers. Worryingly, their incidence has been rising. For over a decade, the first-line standard of care (SoC) regimen for advanced BTC (aBTC) was Gemcitabine-Cisplatin. Recently, two phase III clinical trials showed that adding immune checkpoint inhibitors (ICIs) to the Gemcitabine-Cisplatin chemotherapy backbone results in a modest improvement in overall survival (OS) over Gemcitabine-Cisplatin alone. This led to FDA approval, and thus Gemcitabine-Cisplatin-ICI is now the new first-line SoC. There is limited real-world data on the efficacy and safety of this regimen, particularly in Asian countries.

Method: We conducted a single-centre retrospective chart review to evaluate the real-world efficacy and safety of the new first-line Gemcitabine-Cisplatin-ICI regimen, as well as that of subsequent second-line treatment regimens for patients with aBTC

Results: 42 patients with aBTC who received first-line Gemcitabine-Cisplatin-ICI at the National Cancer Centre Singapore (NCCS) were identified between May 2020 to October 2024. Median follow-up duration was 9.0 months. Median OS of the study population was 20.9 months (95% CI, 8.7-25.9), while median progression-free survival (PFS) was 5.5 months (95% CI, 3.8-8.7). The objective response rate (ORR) was 31.6%. 59.5% of patients received second-line chemotherapy after progression on Gemcitabine-Cisplatin-ICI, for which median OS was 9.3 months (95% CI, 4.6-19.5) and median PFS was 2.5 months (95% CI, 1.8-19.5). The overall ORR for all second-line regimens was 16.7%. Adverse events (AEs) of any grade occurred in all patients, with 51.2% experiencing grade $\geq$3 AEs while on first-line treatment and 22.2% while on second-line treatment.

Conclusion: Our real-world experience demonstrated similar or better efficacy and safety of first-line Gemcitabine-Cisplatin-ICI and subsequent second-line regimens in patients with aBTC compared to literature. These findings support continued use of these regimens for patients with aBTC.

PO8-12-YI
Enhancing liver biopsy accuracy with contrast-enhanced ultrasound: insights from Cracow University Hospital

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Background and aims: Popularization of imaging techniques that visualize abdominal cavity, especially ultrasound (US), have resulted in the higher detection rate of the liver lesions. If other imaging results (magnetic resonance (MRI) or computed tomography (CT) are inconclusive percutaneous liver biopsy should be considered. This procedure is performed mostly with US assistance. However, there are situations in which B-mode US fails to deliver necessary information to ensure safe and efficient biopsy. Modifications of US-guided biopsy emerged to address this problem, such as fusion imaging and contrast-enhanced ultrasound (CEUS). Limitations of fusion-guided biopsy include prolonged time of data processing and difficulties in achieving optimal overlap of images. On the other hand CEUS enhances lesion visualization, but is devoid of mentioned limitations - it is feasible, rapid and requires no additional processing. Moreover, it allows to detect neuroendocrine tumors or metastasis due to assessment of wash-out phenomenon and early arterial phase that create distinguishable patterns. Furthermore, because of necrotic – non-necrotic tissue distinction CEUS can increase efficiency of biopsy by collecting specimen of high quality.

Method: In analyzed medical records from database of Cracow University's Hospital from 08.2022 to 08.2024 there were 190 US-guided liver biopsies performed, including 143 biopsies due to the liver lesion detected on MRI or CT scan. 37 (25.9%) of those biopsies required switch to CEUS because of questionable imaging situation – in particular in case of poorly visible lesions or possible necrotic areas.

Results: According to Cracow University's Hospital database, the diagnostic sensitivity and pathological diagnostic rate of CEUS-guided liver biopsy in our center is around 83.8%, with diagnostic specificity 100%.
It is also worth mentioning that, in 2-years time span there was 1 situation in which the CEUS image allowed the ultrasonographer to initially determine the benign character of lesion, therefore rendering biopsy completely unnecessary.

Conclusion: According to the published studies, diagnostic sensitivity fluctuates between 92 - 96%, whereas pathological diagnostic rate 96.4-100%. In comparison, the same studies estimate the diagnostic sensitivity of a B-mode US assisted biopsy to be between 75 - 92% and pathological diagnostic rate 82.2 - 100%. The disconcordance between our results and literature findings can be explained mostly by the difference in chosen CEUS indications – in other studies they are the same as for US-guidance followed by randomization, but in our case CEUS was chosen when the B-mode US did not deliver sufficient information to perform safe biopsy.

PO8-14-YI
Fibroscan role in determining the patients who are at high risk for developing HCC in 1000 cirrhotic Egyptian patients

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Background and aims: Hepatocellular carcinoma (HCC) is common primary malignancy of the liver and one of the most frequent cause of death in patients with liver cirrhosis. Nowadays, liver stiffness measured noninvasively by transient elastography has been reported to be well correlated with histologically assessed liver fibrosis stage. The aim of this study was to study the role of ultrasound elastography (FibroScan) in early detection of HCC in cirrhotic patients as well as verifying whether it could be used as a tool for identifying cirrhotic patients who are at high risk of developing HCC.

Method: This study was conducted on 1000 patients with cirrhosis of both sexes. The studied patients were classified into: group I which included 500 patients with cirrhosis and HCC diagnosed by ultrasound examination and confirmed by triphasic computed tomography and group II which included 500 patients with cirrhosis and without evidence of HCC diagnosed by clinical, laboratory and ultrasound examination. Patients with morbid obesity, ascites, hepatic metastatic lesions and patients with HCC beyond Milan criteria were excluded from this study. Tumour characteristics were assessed. Tumor staging was done using Okuda, CLIP, VISUM and Tokyo staging systems. Transient elastography was done for all patients and the results were expressed in kilopascal.

Results: Patients with HCC had a mean age of 57.3 years old, while cirrhotic non-HCC patients are younger with a mean age of 51.4 years old. Also HCC commonly presented in males (86%) more than females (14%). Liver stiffness was significantly higher in HCC patients compared to cirrhotic patients. The sensitivity and specificity in diagnosis of HCC were 76% and 87% respectively at cut-off of 30.5 kpa with 91.8% accuracy. Fibroscan has a positive significant correlation with tumour size (P<0.001), Child-Pugh (P<0.001), Okuda classification (P<0.001), CLIP staging (P<0.001) and Tokyo classification (P<0.001) among HCC patients. It was found that likelihood of HCC risk was correlated with increase of liver stiffness. At liver stiffness of 25-30 kpa the probability of HCC is 93% so, these patients should undergo close follow up. Patients with stiffness ≥30 kpa had HCC.

Conclusion: According to this study, fibroscan has an important role in early detection of HCC in cirrhotic patients with cut off 30.5 Kpa. Cirrhotic patients with liver stiffness ≥30 kpa are most likely to have HCC. Cirrhotic patients with liver stiffness ≥25 kpa need close follow up as they are at high risk to develop HCC.

PO8-15

Advancing diagnostic adequacy and patient safety in liver lesions: The role of EUS-guided liver biopsy in modern oncology

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Background and aims: Endoscopic ultrasound-guided liver biopsy (EUS-LB) using 19–22-gauge needles is a promising alternative to traditional liver biopsy. Concerns exist about the adequacy of these samples, which are thinner and may undergo more post-procedural fragmentation compared to percutaneously obtained biopsies. This study evaluates the clinical utility of EUS-LB, specifically in relation to the diagnostic adequacy in mass lesions.

Method: We retrospectively reviewed 50 consecutive EUS-LB procedures performed at our institution for liver masses from April 2018 to April 2024. Data collected from the patient chart included demographics, clinical indications, laboratory results, procedural details, and pathological diagnoses. Biopsies were obtained using 22-gauge heparin-primed Boston Scientific FNB Acquire needles without rapid on-site evaluation. Tissue was considered adequate if it was sufficient for diagnosis, including any necessary ancillary workup.

Results: Of the 50 biopsies, 47 (94%) were adequate for diagnosis, of which 4 (8.5%) were benign (2 focal nodular hyperplasia, 1 hepatic adenoma, and 1 hemangioma). Of the malignant lesions (43), 12 (28%) represented primary liver malignancies (5 hepatocellular carcinomas, 4 cholangiocarcinomas, and 1 undifferentiated carcinoma). Liver metastases were observed in 33 cases (14 from pancreas, 3 from upper gastrointestinal tract, 3 from extrahepatic cholangiocarcinoma, 1 from colorectal primary, and 2 from neuroendocrine neoplasms, site unknown at the time).
In 15 cases with a metastatic diagnosis (45%), concurrent biopsies from the primary site were performed during the same endoscopic procedure, thereby allowing tumor diagnosis and staging at the same time.

Conclusion: EUS-LB allows for simultaneous tumor sampling from primary gastrointestinal sites as well as sampling liver metastasis if accessible via EUS. This procedure for mass lesions at our institution has a high diagnostic adequacy rate of 94% as seen in our study. Other advantages of this technique are the ability to examine and sample for gastrointestinal mucosal pathology, including incidental pancreatic pathology (such as a cyst) in the same setting, reducing the need for multiple procedures. EUS-LB provides a minimally invasive and highly efficient diagnostic tool, offering significant advantages over traditional biopsy techniques in the context of primary gastrointestinal malignancies with suspected liver metastasis.

PO8-16
Morphomolecular proliferative disease and microvascular invasion characterize PET-CT positive hepatocellular carcinoma cases: A single-center pilot study

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Background and aims: Positron emission tomography (PET) – computed tomography (CT) is a helpful diagnostic tool for excluding metastatic disease in patients with hepatocellular carcinoma (HCC). However, PET-CT is rarely used for the diagnosis of intrahepatic lesions in HCC, due to its low sensitivity, which is associated with relatively elevated Standard Uptake Value (SUV) in both normal and cirrhotic liver. PET-CT positivity for primary HCC has been associated with microvascular invasion and more aggressive tumour behaviour. We aimed to study the association between the SUVmax of primary HCC in PET-CT and HCC morphomolecular classification based on liver biopsy.

Method: Twenty-eight patients (22 males, 7 with diabetes, 17 with cirrhosis, 7 of viral aetiology, 9 with varices, 15 classified as ALBI-I, BCLC stage: 7 A, 11 B, 10 C) were included in the study. All patients had primary HCC lesions and underwent ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET-CT examination and imaging-guided liver biopsy. We studied the SUVmax of all histologically proven HCCs. We divided HCCs into proliferative (pHCC, n=14) and non-proliferative (npHCC, n=14).

Results: Sixteen cases were PET-positive and the rest 12 PET-negative. Microvascular invasion was observed in 13 pHCC patients (92.8%) and in only 2 npHCC patients (14.3%) ($p < 0.001$). Baseline characteristics of the two groups were comparable for all variables studied (gender, age, diabetes, cirrhosis, etiology, varices presence, Albumin-Bilirubin/ALBI grade, α-fetoprotein serum values). Median SUVmax in pHCCs was significantly higher compared to npHCCs (8.5 vs. 4, respectively, $p = 0.001$). In multivariate analysis, SUVmax emerged as the only significant factor differentiating between the two groups ($p = 0.033$, OR = 2.5, 95% C.I. 1.077-5.940). SUVmax was strongly correlated with pHCC (Pearson $r = 0.52$, $p = 0.005$). SUVmax of 5.15 presented a sensitivity of 85.7% and specificity of 78.6% for the prediction of pHCC presence (AUROC 0.86, $p = 0.001$). In the subgroup of BCLC-A/B patients (n=18), microvascular invasion was detected in 7/8 (87.5%) with PET-positive HCC compared to 2/10 (20%) with PET-negative HCC (p=0.004). In BCLC-A/B patients with microvascular invasion, 8/9 patients (88.9%) presented with pHCCs, whereas absence of microvascular invasion presented only in patients with npHCCs (9/9, 100%) ($p < 0.001$).

Conclusion: Our pilot study highlights a significant relationship of SUVmax with histologically confirmed proliferative HCC, a finding requiring further investigation. PET positivity in primary HCC may be related to specific HCC histological/molecular subtypes and/or presence of microvascular invasion.

PO8-17-YI
Effects of portal vein thrombosis on morbidity and mortality in hepatocellular carcinoma

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Background and aims: It was aimed to investigate the effects of portal vein thrombosis (PVT) (tumor thrombosis) on the morbidity and mortality of patients with hepatocellular carcinoma (HCC).

Method: This is a single-center and retrospective study in a tertiary university hospital, and patients' data were accessed from the hospital registry system.

Results: There were 118 HCC patients in this study, and 85 (72%) were men. The mean age was 68.9 ± 10.4 years, the mean duration of chronic liver disease was 149.5 ± 102.6 months, the mean age of disease when HCC was diagnosed was 95.5 ± 100.5 months, and the time from HCC diagnosis until death was 31.5 ± 27.5 months.
22 patients had PVT (18.6%) and all were tumor thrombosis. HCC patients with PVT were younger (p = 0.021). There was no relationship between gender and PVT (p = 0.331). The mean total tumor diameter was 39.9 ± 32.5 mm. Total tumor size was larger in those with PVT (p = 0.037). 15 patients (12.7%) had distant metastasis at the time of HCC diagnosis, and there was no relationship between metastasis and PVT (p = 0.118). 25 patients (21.2%) had ascites and 6 (5.1%) had hepatic encephalopathy (HE). While there was no relationship between ascites and PVT, a significant relationship was seen between HE and PVT (p = 0.002). CRP and ALBI scores were higher in those with PVT (p = 0.013, p = 0.027, respectively).
Local treatments were performed in 110 (93.2%) patients, and 8 (6.8%) patients were not suitable for local treatment. Transarterial chemoembolization was performed in 66 patients (55.9%), transarterial radioembolization in 23 (19.5%), and radiofrequency ablation in 21 (17.8%). The non-compliance rate with local treatment was significantly higher in patients with PVT (p = 0.001). 32 (27.1%) patients also were treated with systemic therapy. There was no relationship between receiving systemic treatment and PVT (p = 0.107).
61 (51.7%) patients died during follow-up. The time from HCC diagnosis until death was significantly shorter in patients with PVT than those without PVT (p = 0.001). 25 (21.2%) patients died within 12 months of HCC diagnosis. There was a significant relationship between PVT and both overall mortality and mortality within the first 12 months (p = 0.008, p = 0.001, respectively).

Conclusion: The chance of local treatment is low in HCC patients with PVT, and survival is significantly reduced in HCC patients with PVT.

PO8-19
Clinical and epidemiologic characterstics of patients with hepatocellular cancer in `Sub-Saharan Africa: A five year single-center retrospective study

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Background and aims: Hepatocellular carcinoma (HCC) is a prevalent primary malignant neoplasm originating from hepatocytes, accounting for approximately 80% of all liver cancers. Globally, HCC ranks as the 6th most common malignancy and stands as the 4th-leading cause of cancer-related mortality. The incidence of HCC exhibits significant geographic variability. HCC emerges as a multifaceted disease entity with diverse potential aetiologies, intricately linked to numerous risk factors.

Method: The study was conducted at Tikur Ambessa Specialized Hospital during the period from April 2016 to March 2022. Employing a descriptive cross-sectional study design, we focused on adult patients diagnosed with HCC. The source population encompassed all adults within the study area who received an HCC diagnosis, while the study population comprised those diagnosed with HCC during the study period.

Results: In our study among the 145 diagnosed cases, 106 were male and 39 were female, resulting in a male-to-female ratio of 3:1. The age range spanned from 18 to 91 years, with a median (IQR) age of 55 (40-63). Hepatitis B virus (HBV) emerged as the leading cause of HCC, accounting for 32.41% of cases, while hepatitis C virus (HCV) was found in 19.31% of HCC patients. HBV-HCV co-infection was identified in three patients. A substantial proportion (50.34%) of cases remained of unknown or other aetiology. Alcohol use was reported in 34.48% of HCC patients, and 11.03% of patients had diabetes. These findings underscore the complexity of HCC and highlight the need for targeted prevention strategies and surveillance efforts.

Conclusion: Most HCC patients presenting at Tikur Ambessa Specialized Hospital were not identified through surveillance efforts. Furthermore, most HCC cases do not meet criteria for either surgical or non-surgical therapeutic interventions. Health facilities should prioritize policies aimed at preventing HBV/HCV transmission and implementing targeted surveillance for high-risk groups.

PO8-21-YI
High serum interleukin-6 and interleukin-8 levels are associated with tumor burden and poor prognosis in patients with hepatocellular carcinoma

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Background and aims: Interleukin (IL)-6 and IL-8 are inflammatory response mediators that promote angiogenesis, which is essential for tumor growth and metastasis. We aimed to investigate the association between circulating IL-6 and IL-8 levels and the clinicopathological features and prognosis of patients with hepatocellular carcinoma (HCC).

Method: A total of 285 patients (median age: 68, IQR 60–75 years; males: 231, 81.1%) with a new diagnosis of HCC were retrospectively enrolled. Liver disease etiology was HCV in 130 (45.6%) and MASLD in 155 (54.4%). Most patients had early stage HCC (BCLC: 0/A, n = 184, 64.6%); patients with BCLC D were excluded from the analysis. Serum IL-6 and IL-8 were quantified in serum samples collected at HCC diagnosis by multiplex immunoassays (Bio-Plex® 200 System, Bio-Rad, USA). Primary end-point was overall survival (OS).

Results: Median cytokines values stepwise increased according to BCLC stage (IL-6: from 3.45, 1.95–5.62 pg/mL at stage 0 to 6.59, 3.57–11.04 pg/mL at stage C, $p < 0.001$; IL-8: from 30.34, 18.25–66.32 pg/mL at stage 0 to 69.22, 28.11–199.59 pg/mL at stage C, $p < 0.001$). Patients with IL-6 > 4.28 pg/mL (Shao et al. 2017) showed poorer OS (27.1, 95%CI 20.8–32.9 months) than those with IL-6 ≤ 4.28 pg/mL (median OS: 27.1 vs 50.1 months, $p < 0.001$); similarly, patients with IL-8 > 17.60 pg/mL (Ren et al. 2003) had poorer OS than those with IL-8 ≤ 17.60 pg/mL (median OS: 27.9 vs 57.2 months, $p = 0.001$). At multivariate analysis, IL-6 > 4.28 pg/mL (HR = 1.57, 95%CI 1.06–2.32) and IL-8 > 17.60 pg/mL (HR = 1.79, 95%CI 1.11–2.90) resulted independent predictors of OS irrespectively from BCLC stage (HR = 2.28, 95%CI 1.86–2.81). Finally, the combination of IL-6 and IL-8 measurement allowed to stratify patients into 3 risk categories with different OS: low-risk (IL-6 ≤ 4.28 pg/mL and IL-8 ≤ 17.60 pg/mL; median OS: 109.5 months), medium-risk (IL-6 > 4.28 pg/mL or IL-8 > 17.60 pg/mL; median OS: 34.3 months), and high-risk (IL-6 > 4.28 pg/mL and IL-8 > 17.60 pg/mL; median OS: 26.7 months) ($p < 0.001$).

Conclusion: In patients with HCC, circulating IL-6 and IL-8 values were associated to tumor burden and their combined use may provide prognostic information and allow for personalized treatment strategies. *Project PNC 0000001 D[3] 4 Health, The National Plan for Complementary Investments to the NRRP, funded by the European Union – NextGenerationEU.*

PO8-22
Clinical characteristics and outcomes in patients diagnosed with hepatocellular carcinoma receiving resection or ablation in England

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Background and aims: Hepatocellular carcinoma (HCC) is a difficult cancer to treat with a high incidence rate of recurrence and de novo tumours. This study describes characteristics, outcomes and treatment pathways in patients with HCC, who received a potentially curative ablation/resection as part of their first treatment after diagnosis.

Method: Patients diagnosed with HCC (ICD10 code C220 and morphology code 8170) between 01/01/2015 - 31/12/2021, aged ≥18 years, were selected from the National Cancer Registration Dataset, England. Ablation/resection, locoregional (trans arterial chemoembolization/trans arterial embolization/etc.), transplant and systemic treatments were identified using the Hospital Episode Statistics (HES) and Systemic Anti Cancer Therapy Datasets. Those receiving ablation/resection within 30 days prior to HCC diagnosis, or any time after were included if this was the first treatment, or if the only prior treatment to ablation/resection was locoregional treatment (up to 12 weeks prior). First ablation/resection was index date. Follow-up (FU) ended 05/04/2024. Comorbidities up to 5 years prior to diagnosis were extracted from HES. Subsequent treatments were summarised. The Kaplan-Meier method estimated time to next treatment (TTNT) and overall survival (OS) from index.

Results: Of 3331 patients with HCC who received ablation/resection, 2861 (85.9%) received it as first treatment; 105 (3.7%) had locoregional treatment within 12 weeks prior to index. At index, 1449 (52.4%) patients received ablation, 1327 (46.4%) resection and 35 (1.2%) received both. Median time from diagnosis to index was 1.8 months (interquartile range (IQR): 0 - 3.7). Median age at diagnosis was 68 years (IQR: 60 - 74); 2224 (77.7%) were male. In all, 1142 (39.9%) had diabetes, 1056 (36.9%) cirrhosis, 601 (21.0%) alcohol-related liver disease, 278 (9.7%) non-alcohol-related fatty liver disease, 181 (6.3%) fatty liver disease, and 24 (0.8%) and 5 (0.2%) hepatitis B and C, respectively. Median FU from diagnosis was 40.9 months (IQR: 24.6 - 60.2). During FU, 1207 (42.2%) patients received a next treatment. Median TTNT and OS were 28.1 months (95% CI: 26.2, 30.1) and 62 months (95% CI: 58.2, 66.5), respectively.

Conclusion: In this HCC cohort with high prevalence of comorbidities, 42.2% started a new treatment during FU after their initial ablation/resection, with TTNT numerically shorter than OS. This suggests a high recurrence rate, requiring further research into maximizing the opportunity for curative intervention.

PO9-02-YI
FIB-4 in predicting hepatocellular carcinoma during viral hepatitis: A novel indication for an old tool

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Background and aims: Hepatocellular carcinoma (HCC) is a severe outcome during liver disease with limited treatment options when diagnosed lately. It is the leading cause of death in patients with chronic hepatitis, particularly secondary to chronic viral hepatitis. Screening scores are continuously being developed to optimize early diagnosis. The aim of this study was to investigate the correlation between FiB-4 score and HCC risk during treated chronic viral hepatitis in Tunisian patients.

Method: We conducted a single-center retrospective study analyzing data from patients with chronic viral hepatitis B and C. Patients with viral co-infection or associated nonalcoholic steatohepatitis were excluded. FIB4 score was calculated for each patient at presentation.

Results: In total, 88 consecutive patients were enrolled: 42 with chronic hepatitis B and 46 with chronic hepatitis C. The mean age at diagnosis was 51.5 ±12 years with a sex-ratio M/F of 1.31. At baseline, 37.2% of patients had already developed cirrhosis. Virological response was achieved in 92% of the population. HCC occurred in 16% (N=14) of patients after a mean follow-up period of 79. The stage of HCC was classified as 'A', 'B', 'C' and 'D' according to BCLC clasification in 28.5%, 25%, 43% and 0.7% respectively. Univariate analysis identified: older age ($p=0.02$), low platelet count ($p<0.0001$), cirrhosis ($p<0.0001$), high bilirubin level ($p=0.028$) and high Fib-4 ($p=0.01$) as factors associated with the occurrence of HCC. When analyzing the receiver operating characteristic (ROC) curve, AUC of FIB4 score in predicting HCC was 0.845 (95% CI: 0.730–0.960, $p<0.0001$). The corresponding FIB-4 cut-offs for 90% sensitivities was 1.67, while the cut-offs for 90% specificities was 3.8.

Conclusion: In our study, high FIB-4 score was associated with increased HCC risk in patients with viral hepatitis. FIB-4 index is based on routinely clinical and biological data and showed highly predictive performances in predicting HCC allowing better stratification.

PO9-03-YI

Local control and recurrence patterns after stereotactic irradiation delivered in more than 4 fractions for hepatocellular carcinomas and liver metastases

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Background and aims: Stereotactic Body Radiation Therapy (SBRT) is a safe and effective treatment for liver metastases or hepatocellular carcinoma (HCC) with a dose-response relationship for local control (LC). Proximity to organs at risk (OAR) often requires dose de-escalation. This study evaluated LC and recurrence patterns in patients administered hepatic SBRT in more than 4 fractions due to dosimetric constraints.

Method: This retrospective study included 33 patients treated with SBRT (Cyberknife®) in more than 4 fractions for HCC or liver metastases, between January 2011 and December 2019. Patients were ineligible for treatment in 3 or 4 fractions due to OAR proximity. Recurrence patterns were analysed according to the volume shared between recurrence and initial target or treatment isodose volumes.

Results: The primary dose ranged from 35-50 Gy delivered in 5 to 7 fractions for the treatment of HCC (39%) or liver metastases (61%) mainly secondary to colorectal cancer (40%).
LC rate was 64%, with 12 patients showing recurrence volume overlap with the initial target volume or treatment isodose. In-field recurrence occurred in only 12.5% of patients with most relapses being out-of-field. No grade ≥3 events were reported.

Conclusion: Despite dose reductions to spare OAR, SBRT showed satisfactory LC with low toxicity. Out-of-field recurrence remains the most common pattern identified and likely related to underlying disease. Prospective data are necessary to determine whether preserving dose while reducing planning target volume (PTV) coverage could enhance LC.

PO9-04-YI
Tyrosine Kinase Inhibitors were well-tolerated among patients with different etiologies of advanced HCC with lower survival in non-viral patients

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Background and aims: Sorafenib is one of the hepatocellular carcinoma (HCC) first-line therapy for Barcelona clinic liver cancer (BCLC) stage B-three or advanced stage-C after adequate multidisciplinary assessment if immunotherapy is not available. Regorafenib is a second-line for sorafenib non-responders. We aimed to study outcome of HCC patients treated with Sorafenib and Regorafenib and the influence of the underlying etiology

Method: This retrospective multicenter study recruited patients with sorafenib-treated HCC (12/2016 to 4/2023) till death or the study end (2/2024) from six tertiary care canters in Egypt. Time to progression (TTP) and overall survival (OS) were recorded. We evaluated OS predictors, The impact of underlying etiology, and tolerance for sorafenib for more than 6 months

Results: This study included 706 patients (622 (88.1%) had HCV-related, 15 (2.1%) had HBV-related, and 69 (9.8%) had non-viral-related HCC). The median age was 62 years with male predominance (76.1%), 98% had cirrhosis with Child-Pugh-A in 96.5%. 59.5% had BCLC-stage-B and others had BCLC-C. The commonest Sorafenib adverse events (AEs) were jaundice (30.9%), fatigue (24.6%), anemia (19.3%), and elevated liver enzymes (15.4%)**.**
By the end of the study,379 (53.7%) patients died. The median duration of Sorafenib therapy was 240.00 days. The disease control rate (DCR) (partial response and stable disease) was 66.56%, median OS 314.00 (146.00 -601.00) days, median TTP 180.00 (90.00- 330.00) days. The independent factors affecting mortality by COX-regression: baseline serum AST ($p < 0.001$, Hazard ratio (HR) 1.005, 95.0% CI (1.002 - 1.007)), HCC size (p 0.007,HR 1.039,95.0% CI (1.010 - 1.068)), hepatic vein thrombosis (p

0.013,HR 1.739,95.0% CI (1.125 -2.688)), jaundice development (p < 0.001,HR 2.192,95.0% CI (1.756 -2.737)), shifting to Regorafenib (p < 0.001,HR 0.340,95.0% CI (0.205 -0.566))
HCCs developed on top of non-cirrhotic liver in 7.1% of HBV and 7.1% of non-viral and 1.3% of HCV aetiology. Adverse events, TTP and tumor response didn't differ with the underlying etiology. Median OS was lower in non-viral-related than HCV-related HCC (218.00 versus 326.50 days,p 0.048)**.**
Patients who continued sorafenib above 6-months had lower AFP, more BCLC-B, less AEs and better 3-months tumor response and longer OS (680 vs 198days,p 0.001). 68 (9.6%) patients shifted to Regorafenib. DCR 70.9%. The most prevalent AEs were mild elevation of liver enzymes, decompensation and hypertension. The most common cause of discontinuation was decompensation (64.2%)

Conclusion: OS is lower in non-viral Sorafenib-treated compared with viral-related HCC and Sorafenib was well-tolerated among different HCC etiologies

PO9-05-YI
Neoadjuvant chemotherapy as a tool of systemic control following portal vein embolization before major liver resection in patients with locally advanced intrahepatic cholangiocarcinoma

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Background and aims: A substantial proportion of patients with intrahepatic cholangiocarcinoma (ICC) present with locally advanced disease with macroscopic vascular involvement (MVI) or multiple tumors. In such cases, the technical feasibility of major liver resection is achieved through preoperative portal vein embolization (PVE). Taking into account that locally advanced ICC is already a borderline resectable disease, the waiting time following PVE further poses the risk of tumor progression. In light of emerging evidence of neoadjuvant chemotherapy benefits in ICC, with the present study, we aim to investigate the safety and feasibility of NAC in patients with locally advanced ICC who underwent major liver resection following PVE.

Method: We retrospectively analyzed the prospectively collected data on patients with locally advanced ICC, who received NAC following PVE and subsequently underwent major liver resection. The locally advanced ICC included those with macroscopic vascular invasion, multiple tumors, and clinically positive regional lymph nodes. Cases with extrahepatic metastases and distant clinically positive lymph nodes were considered definitely unresectable.

Results: 5 patients who underwent the PVE procedure before major liver resection received NAC. The patients' age was 42-76 y (median 60,4 y). The NAC regimens included Gemcitabine plus Cisplatin, Gemcitabin plus Oxaliplatin, Gemcitabine. The number of NAC courses was 2 - 4. There were no cases of NAC withdrawal due to toxicity. The time from diagnosis to surgery was 54 – 105 days (median 71,2 d). The types of resection included right hemihepatectomy, right hemihepatectomy with segment 1 resection, and right trisectionectomy. In cases of MVI, there was 1 case of right portal vein invasion and 1 case of right hepatic vein invasion. On the final histological report, there were 4 cases of ypT2N0 stage and 1 case of ypT2N1 stage. In all cases, R0 resection was achieved. In all cases lymphovascular invasion was present, and perineural invasion with vascular invasion was observed in 3 cases. A pathological partial response was observed in all cases. The postoperative course was unremarkable.

Conclusion: Neoadjuvant chemotherapy could be safe and feasible option of local control for patients with locally advanced ICC waiting for major liver resection following PVE.

PO9-06
Treatment with combination transarterial chemoembolization and Lenvatinib plus sequential microwave ablation have improved the survival of Barcelona clinic livercancer stage B2 in Bangladesh

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Background and aims: Our aim was to investigate the efficacy and safety of Transarterial chemoembolization (TACE) combined with Lenvatinib plus sequential Microwave Ablation(MWA) for the treatment of patients with large Hepatocellular Carcinoma (HCC) Barcelona clinic liver cancer stage B2 (beyond upto 7 criteria)

Method: This multi center study included 135 cases of Stage B2 from January, 2015 to January 2022.Age range:28-82 yrs;102 males,32 females;112 child A,23 child B cirrhosis; Tumor size: 7-12 cm in maximum diameter;Tumor number:maximum 5 in number.We included only those,whose atleast 50% liver parenchyma were free from malignant tumors. We divided the patients in two groups. i) TACE-Lenvatinib-MWA(TLM) group (n=47)and ii) TACE-MWA(TM) group(n=88) .We again subdivided each of the groups based on size of the biggest tumor. a) 7-8 cm tumor group b)8-10 cm tumor group. C) >10cm Tumor group.In TLM group, we started Lenvatinib 7 days after TACE. MWA was performed 21 days after Lenvatinib administration. After recovery from MWA, we restarted Lenvatinib and continued till recurrence of tumor. In TM group we did MWA 7 days after TACE. We re-ablated the recurred tumors. The progression-free survival (PFS), cumulative overall survival (OS) and treatment related complications were compared

Results: Technical success of combined transarterial chemoembolization and Microwave Ablation was achieved in all patients (100% either in single or multiple sessions). Follow-up MRI/CT was done to assess the treatment response after one month. The median follow up period was 48.5 months. The TLM group had longer PFS than the TM group (median, 17.32 vs. 6.3 months, $p < 0.001$; median.1,3,5 year cumulative over all survival of patients in TM group: 7-8 tumor subgroup: 96%, 72 %,42%; in 8-10 cm subgroup:87%, 45%,18%;in more than 10 cm tumor sub group: 48%,24%,3% and in TLM group: 7-8 tumor sub group: 95%,77%,51% ;in 8-10 tumor sub group: 92%,59%,32%;in more than 10 cm tumor sub group:61%, 34%,9%. There was no treatment related death. Minor complications like pain, fever, nausea occurred in 13% cases while major complications such as Hepatic failure, hemorrhage, pleural effusion occurred in 1% cases.

Conclusion: TACE-Lenvatinib-MWA can prolong the progression-free survival and over all Survival of patients with large hepatocellular carcinoma beyond up-to-seven criteria with high safety rateo

PO9-08-YI
Primary Clear cell adenocarcinoma of the liver and the risk of multiple primary gastrointestinal malignancies

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Background and aims: Primary clear cell adenocarcinoma is considered one of the rarest types of malignant tumors of the liver. The frequency of Primary clear cell carcinoma of the liver (PCCA) varying between 2.2% and 6.7% among HCCs reported in the published studies. Developing gastrointestinal (GI) Second primary malignancies (SPMs) after PCCA is a sporadic event reported in the literature. There are no studies discussing the association between GI SPMs and PCCA. So, this study aimed to cover this gap and provide updated evidence to the literature about this rare type.

Method: Data were extracted using the Surveillance Epidemiology and End Result (SEER) database. We used an MP-SIR session with multiple outcome analysis and a latency exclusion period of two months to assess the risk of GI SPMs in patients diagnosed with primary PCCA. Standardized Incidence Ratio (SIR) was calculated as observed/expected (O/E), and excess absolute risk (EAR) is per 10,000. Significance was achieved at 0.05 with a 95% confidence interval (CI).

Results: There was increased risk for GI SPMs after PCCA in the 6–11 months interval with an O/E of 2 ($P<0.05$, EAR = 22.16), while along 10+ years of follow up, the O/E was 1,27($P<0.05$, EAR =6.86). Small intestine SPMs after PCCA among who received no chemotherapy had an EAR = 0.47(O/E = 1.63, $P<0.05$, 95%CI: 1.12-2.3) while those who received chemotherapy had an O/E= zero. The middle age had the risk of Gi SPMs (O/E =1.26, $P<0.05$, EAR = 1.42) compared to the young age (O/E=zero, $P>0.05$) and elderly (O/E of 0.99 ($P>0.05$).

Conclusion: patients diagnosed with liver PCCA are at increased risk of developing GI SPMs for small intestine and between 6–11 months interval. The middle age and the patients who received no chemotherapy are more susceptible to suffer from GI SPMs. all middle-aged and elderly with liver PCCA to undergo routine follow-ups starting from the time of diagnosis to minimize the burden of SPMs among cancer survivors.

PO9-10
Feasibility and effectiveness of liver transplantation following immunotherapy in patients with hepatocellular carcinoma

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Background and aims: Immunotherapy is an attractive strategy for down-staging/bridging before liver transplantation (LT). This multicentre study reports the results of the patients transplanted in France in this context.

Method: All patients in France who underwent LT for HCC after receiving immunotherapy were identified and included in the study. Clinical/biological/radiological data were collected for each patient at the beginning/end of immunotherapy and before LT. The primary endpoint was the tolerance and effectiveness of LT. All statistical analyses were performed using R software version 4.2.0.

Results: Twenty-one patients [17 males; median age 62 years (57-64)] underwent LT for HCC after immunotherapy. Sixteen patients (76.2%) received Atezolizumab *plus* Bevacizumab [median 8.5 cycles (4.7 – 14)], and at baseline, fourteen out of 21 patients (66.7%) were BCLC-B. HCC was multi-nodular in 17/21 (81%) cases, with a median size of the largest nodule of 36 mm (21-60). At the end of immunotherapy, most patients were Milan-In (57.1%) criteria, while the AFP score was >2 in only 2 cases (9.5%) (p = 0.003). The interval between the last immunotherapy cycle and LT was 5.1 (2.7-9.3) months. All patients except three received standard immunosuppressive treatment. Five patients (23.8%) died, two had an allograft rejection, and two patients (10.5%) had an HCC recurrence after LT. On explant pathology, six patients (28.6%) had no tumor residue. The R3-AFP score stratified patients into three at very low (14.3%), eight at low (38.1%), and ten at high (47.6%) risk.

Conclusions: LT following immunotherapy is feasible in selected patients with HCC and has an acceptable risk of rejection. However, the high immediate mortality observed requires further exploration in a prospective series.

PO9-12-YI
A new machine learning-based algorithm to identify patients with hepatocellular carcinoma

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Background and aims: With chronic liver disease emerging as a global healthcare challenge, hepatocellular carcinoma (HCC) is one of the fastest growing causes of cancer-related death. This is mainly due to inefficient early-stage detection, suboptimal risk stratification, the limited curative treatments for advanced stages and the underlying impaired liver function. The aim of this study was to develop a machine learning model to identify, through the integration of clinical and laboratory data, patients with HCC from patients with liver cirrhosis (LC).

Method: The population study (n=160) with available clinical and laboratory data, was classified in 2 subgroups of patients, prospectively followed up in our referral center: 72 patients with LC, 88 with HCC. The different groups of patients were used as training set. Using machine learning algorithms, classifiers were created using Random Forest analysis, Random Ferns classification and the Ensemble approach.

Results: Data from the 160 patients with LC and HCC were used to create a classifier using a Random Forest model. This model was then validated on the same dataset with an overall classification error of 0.23125, specifically 0.2778 for LC and 0.1932 for HCC. The same data were then analyzed with a Random Ferns model (accuracy: 0.7312, F1 score: 0.7190, kappa: 0.4632), and an ensemble approach method (accuracy 0.9938, kappa 0.9874). The superlearner was able to classify patients with high sensitivity and specificity.

Conclusion: This machine learning approach identified a new algorithm that could help clinicians to easily identify patients with HCC.

PO9-14-YI

Outcome associated prognostic factors in hepatocellular carcinoma patients undergoing resection: study of clinical, pathological, and serological aspects

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Background and aims: Hepatocellular carcinoma (HCC) is the most common liver cancer, the leading cause of death in patients with cirrhosis, and one of the main causes of cancer related death globally. Surgical resection is one of the curative therapeutic options for early stage HCC; however, high postoperative recurrence rates significantly condition long-term survival. We aimed to evaluate prognostic factors associated with recurrence and mortality in surgically resected HCC patients in our institution.

Method: 116 patients that underwent HCC resection from January 2014 to July 2024 were included. Clinical and liver function data, tumour characteristics, surgical approach, and pathological study (PS) data were collected. 29 patients were excluded (5 incomplete surgery, 6 "ab initio" liver transplant, 6 adjuvant therapies in clinical trials, and 12 follow-up < 1 year post-surgery). 87 patients were included. 80 patients had basal blood sample for study of plasma biomarkers and 44 had cfDNA.

Results: 80.2% were male, median age 65 ± 18 years and median follow-up 39.5 ± 56 months. 94.8% had underlying liver disease, mainly hepatitis C virus infection (47.4%), and 38.8% had cirrhosis. HCC was diagnosed by screening ultrasound in 40.5%; 88% were in early stages (BCLC 0/A), and 99.1% were Child-Pugh A. 81% underwent segmentectomy, and 52.6% had minimally invasive surgery. 60.9% had moderately differentiated HCC, and 34.5% had poor prognostic factors in PS (microvascular invasion and/or satellitosis). 46 patients (39.7%) experienced recurrence, being early (< 2 years) in 34 (29.3%). 24 recurrences (52.2%) happened at not curative stages (BCLC B/C). Overall survival was 75%, and 13.7% had HCC-related death. Factors related to recurrence were intraoperative complications (p = 0.026) and HCC size in PS (40 vs. 30 mm, OR 1.015 [1.001 - 1.030]; p = 0.041). Survival was associated with ALBI A1 (HR 0.164 [0.056 - 0.485]; p = 0.001), minimally invasive approach (OR 0.162 [0.034 - 0.779]; p = 0.023), and curative-stage recurrence (BCLC 0/A) (p = 0.032). Patients with aggressive recurrence (BCLC B/C) had larger tumours (p = 0.009), lower PS differentiation (p = 0.009) and 50% had HCC-related death. Baseline cfDNA was higher in patients with recurrence and death (p non-significative).

Conclusion: Minimally invasive surgery offers a better outcome in HCC. Tumour size and liver function (ALBI) have prognostic impact. More than 50% of recurrences were in non-curative stages, supporting the "ab initio" liver transplant indication for high-risk patients. Additional cfDNA and other plasma biomarkers studies are ongoing.

PO9-15-YI

The impact of various histopathological features and patient characteristics on the outcome of HCC patients: A population based study

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Background and aims: Hepatocellular carcinoma (HCC) is a serious global health concern and accounts for approximately 75–85% of primary liver cancers. Liver cancer ranks fourth in terms of cancer-related mortality and is the sixth most prevalent type of cancer to be diagnosed. There is a lack of thorough information on the precise outcomes and demographics of each histologic variation subtype, despite the fact that the incidence rates of HCC are rising. In order to provide a thorough assessment of all forms, this study will concentrate on their clinical condition and prognosis.

Method: Data were extracted from the Surveillance, Epidemiology, and End Results (SEER) database for HCC patients diagnosed from 2000 to 2021. Patients were grouped according to their clinical characteristics, histological features, and treatments received. We excluded patients with unknown vital status and histological types. SPSS version 27 was used for data analysis. The overall survival (OS) differences between patients were investigated using Kaplan-Meier curves and cox regression hazard function. Mortality prognostic variables were identified using univariate and multivariate analysis.

Results: The study involved 1,450 patients with known hepatocellular carcinoma histology, with clear cell HCC being the most prevalent, particularly in adult and elderly populations, and higher in white males. The study revealed that clear cell and trabecular adenocarcinomas predominantly have localized stages, while fibrolamellar HCC is more frequently staged as regional. . Survival analysis indicated that patients with pleomorphic cell HCC subtype presented with significantly worse overall survival compared to fibrolamellar HCC (AHR for pleomorphic cell variant = 4.895; 95% CI: 1.315-18.22, p = 0.018). Age was a strong prognostic factor, with patients over 60 showing notably decreased survival (AHR = 2.375; 95% CI: 1.147-4.917, p = 0.02). Fibrolamellar and trabecular had the best overall survival among all types.

Conclusion: The HCC histological type affects significantly the survival outcomes to be better in fibrolamellar and trabecular types and plays an important role in the spread pattern of the cancer. Age is still a unique prognostic factor among all types.

PO9-16
ALBI score as a predictor of survival in patients with hepatocellular carcinoma treated by transarterial radioembolization

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Background and aims: To evaluate the predictive factors for survival in patients with hepatocellular carcinoma (HCC) undergoing transarterial radioembolization (TARE). To analyze the efficacy and safety profile of this technique.

Method: Observational, descriptive, single-center study of patients with HCC undergoing TARE between February 2016 and May 2024. Demographic, hepatopathy-related characteristics, overall survival (OS), predictive factors for survival and adverse effects (AEs) associated with the procedure were analyzed.

Results: 50 patients, median age 67 (interquartile range (IQR) 60-77) years; 82% men. The main etiology of liver disease was Alcohol-Related (56%). 54% were hypertensive and 46% had diabetes mellitus (DM). 84% had advanced chronic liver disease (44% had esophageal varices). 86% had a Child-Pugh (CP) class A. As for the ALBI score, 68% had a grade 1 and the remaining 32% had a grade 2.
At diagnosis, HCC was unilobar in 76% of patients, with Barcelona Clinic Liver Cancer (BCLC) B stage in 60% and 84% had an alpha-fetoprotein < 400 ng/mL. Treatment with curative intent was performed in 70%, achieving complete response in 37.1%.

Estimated OS was 94 weeks (95% CI 72-115). According to the variables, ALBI score reached statistical significance (p = 0.007; median 99 weeks in ALBI 1 (95% CI 80-118) and 43 weeks in ALBI 2 (95% CI 7-79)). CP class A vs CP class B approached statistical significance (p = 0.052), as did presenting DM (p = 0.064).
After a median follow-up of 60 (IQR 30-92) weeks, Exitus occurred in 48% of patients.
All AEs recorded were mild and did not require hospital management.
Conclusion: Liver function measured by ALBI score is, in our series, a predictor of survival in HCC patients treated with TARE.
TARE is an effective and safe technique for the treatment of HCC in selected patients.

PO9-17
Assessing the impact of Body Mass Index on Hepatocellular Carcinoma survival following locoregional therapies

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Background and aims: Hepatocellular carcinoma (HCC) is a major cause of cancer-related death worldwide, with locoregional therapies such as radiofrequency ablation (RFA), transarterial chemoembolization (TACE), and transarterial radioembolization (TARE) playing a crucial role in management. Body mass index (BMI) is known to influence cancer prognosis, but its effect on HCC patients treated with locoregional therapy remains unclear. We aimed to evaluate the impact of BMI on survival outcomes in HCC patients treated with locoregional therapies, categorizing patients into overweight (BMI ≥ 25) and non-overweight (BMI < 25) groups, and to assess survival differences between different treatment modalities

Method: A retrospective cohort study of HCC patients undergoing RFA, TACE, or TARE at a tertiary care center was conducted. Patients were stratified into two BMI categories: overweight (BMI ≥ 25) and non-overweight (BMI < 25). Clinical and demographic data were collected, and survival outcomes were analyzed using Kaplan-Meier survival curves. Differences between groups were assessed with the log-rank test, and multivariate Cox proportional hazards regression was performed to adjust for potential confounding variables.

Results: The study included 92 patients, with 63 (68.48%) classified as overweight (BMI ≥ 25) and 29 (31.52%) as non-overweight (BMI < 25). Treatment modalities included RFA (34.78%), TACE (44.57%), and TARE (20.65%). Overweight patients had a median survival of 967 days (95% CI: 608–1739), while non-overweight patients had a median survival of 1406 days (95% CI: 809–1679), with no statistically significant difference in survival (p = 0.206). Among the treatment subgroups, RFA had the highest mean survival estimate (1476.78 days) and median survival of 1759 days (95% CI: 745 to 1853 days), followed by TACE with a mean survival of 1300.98 days and median survival of 965 days (95% CI: 484 to 1636 days), and TARE with a mean survival of 1090.09 days and median survival of 1004 days (95% CI: 447 to 1406 days). A Log-Rank test comparing these treatment groups yielded a chi-square value of 1.5 (p = 0.472), indicating no statistically significant difference in survival among the three therapies.

Conclusion: Overweight status (BMI ≥ 25) was not associated with a statistically significant difference in survival compared to non-overweight status in HCC patients undergoing locoregional therapies. No significant differences in survival were observed between different treatment modalities (RFA, TACE, and TARE). These findings underscore the need for further investigation into the role of BMI and treatment strategies to optimize HCC patient outcomes.

PO9-18-YI
Real world data and outcomes of liver resection in patients with hepatocellular carcinoma

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Background and aims: Liver resection (LR) is a radical curative treatment modality recommended only for early-stage hepatocellular carcinoma (HCC). Despite careful patient selection, cancer recurrence (rHCC) is up to 60% at 5 years. The aim of this study was to gain real-world insight into patient characteristics, tumour recurrence rate as well as overall survival (OS) in patients who had LR as the first recommended treatment modality with/out preceding locoregional therapy (LRT).

Method: Data was retrospectively collected from patients who underwent LR for HCC at King's College Hospital between 2016 and 2023. Wilcoxon rank sum, Fisher's exact or Pearson's Chi-square tests, where appropriate, were used to assess the correlates for rHCC. Kaplan-Meier curves estimated survival and logistic regression models were used to examine the HCC recurrence.

Results: Sixty-six patients were included; 54 (82%) were male, with a median age of 66 (IQR 54 – 73) years at LR. The majority 50 (76%) were non cirrhotic on histology. Twenty-four (36%) had pre-existing MASLD and 23 (35%) had underlying viral hepatitis. At the time of HCC diagnosis, 26 (39%) had LR recommended as the primary treatment modality while 40 (61%) had LRT before undergoing LR. At staging, 42 (64%)/ 11 (17%)/ 13 (20%) were BCLC Stages A/ B/ C respectively with 55 (85%) having a solitary lesion and the largest lesion a median 70 (IQR 43 –110) mm. Patients who had LR as recommended primary modality were more likely to be older ($p = 0.026$), BCLC A ($p = 0.051$) and had shorter time from staging to LR ($p < 0.001$). HCC recurrence occurred in 53% of patients and this rate was broadly equivalent in those with/without LRT prior LR. The median OS at 1yr, 3yrs and 5yrs was 92.6%, 86.0% and 64.5% in those without tumour recurrence vs 78.4%, 63.3% and 58.5% in those with rHCC respectively.

Conclusion: Our real word data shows that LR is an excellent treatment modality across all stages of the HCC. LRT prior LR did not impact in the rate of HCC recurrence which would suggest that LR could be a good initial approach. Despite HCC recurrence the OS rate remains acceptable across the whole spectrum of the disease.

PO9-19-YI
Urban and Rural Differences in Hepatocellular Carcinoma: A Retrospective Analysis from Southern Transylvania

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Background and aims: Hepatocellular carcinoma (HCC) in Transylvania is associated with risk factors such as viral hepatitis or alcohol-related hepatitis. Our aim was to determine the prevalence and biochemical characteristics of HCC among hospitalized patients in Southern Transylvania.

Method: We conducted a retrospective study over a 7-year period. Out of 7,224 patients hospitalized in the Gastroenterology Department of the Clinical County Hospital in Sibiu, 80 were diagnosed with HCC. We categorized these patients based on their area of residence (urban or rural).

Results: The prevalence of HCC among hospitalized patients was 1.1%, with 75% of cases from urban areas. The incidence of HCC in chronic liver disease varied between the two groups: HCC related to hepatitis C virus was more prevalent in urban areas than in rural areas (18.18% vs. 10%). In urban areas, 15.15% of HCC cases were related to alcohol, compared to 10% in rural areas. The mean age of patients from rural areas was 63 years, compared to 64.64 years in urban patients (p=0.3677). Transaminase levels were higher in HCC patients from urban areas (p=0.47 for TGO, p=0.35 for TGP). Bilirubin levels were also higher in urban patients than in rural patients (p=0.37). Gamma-glutamyl transpeptidase levels were higher in urban patients compared to rural patients (p=0.44). Platelet count, coagulation parameters, cholesterol, and triglyceride levels did not significantly differ between the two groups.

Conclusion: The prevalence of HCC among hospitalized patients in Southern Transylvania is approximately 1.1%. Most patients diagnosed with hepatocellular carcinoma (75%) reside in urban areas. In these areas, HCC is more commonly associated with hepatitis C virus infection and alcohol-related hepatitis. Liver cytolysis and cholestasis levels appear to be higher in urban patients at diagnosis, potentially indicating a poorer prognosis for these patients.

PO9-20
Short- And Long-Term Outcomes Of Surgery For Colorectal And Non-Colorectal Metastasis: A Report From University Hospital Center In Lithuania

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Background and aims: Systemic chemotherapy in patients with metastatic colorectal cancer impairs liver regeneration following hepatic resection. The study compares the liver regenerative volume between patients with metastatic colorectal cancer and those with benign liver pathology after hepatic resection. This is the first study in Lithuania aimed at determining the impact of chemotherapy on liver regeneration, as well as the first to investigate the effects of the hepatic regenerative markers HGF and TGF-β1 in humans.
Primary Objective
Overall survival (OS) following liver resection.
Secondary Objectives
Disease-free survival (DFS).
Postoperative morbidity.

Method: Patients were divided into colorectal metastasis (CRC) and non-colorectal metastasis (non-CRC) groups.
The analysis included patients' baseline characteristics—age, sex, primary tumor treatment and origin, metastasis characteristics, surgical treatment, and postoperative complications.
Survival and disease-free survival were evaluated using the Kaplan-Meier method.

Results: The median time to disease progression for CRC was 35.4 months.
The median time to disease progression for non-CRC was 22.5 months.
The 1-year disease-free survival (DFS) rates were 89.4% vs. 76.5%.
The 3-year DFS rates were 64.9% vs. 31.4%.
The median overall survival for CRC was 54.7 ± 4.7 months.
The median overall survival for non-CRC metastases was 48.9 ± 3.2 months.
The 1-year overall survival rates were 89.4% vs. 78.4%.
The 3-year overall survival rates were 72.0% vs. 46.1%.

Conclusion: The short- and long-term outcomes of patients treated at VULSK for liver metastatic disease are consistent with global data.
Perioperative and postoperative outcomes in the study group and the CRLM group undergoing liver resection were similar and showed no statistical differences.
Patients with CRLM who received chemotherapy exhibited lower serum levels of TGFβ-1, decreased low-intensity, and increased high-intensity TGFβ-1 expression in liver tissue.
No differences were found in static liver function markers when evaluating postoperative liver function, except for preoperative alkaline phosphatase levels.
Multivariate linear regression analysis revealed that only the volume of liver resected had a significant impact on liver volume regeneration.

PO9-21
Epidemiology of hepatocellular carcinoma (2016-2023). The epidemic of metabolic associated steatotic liver disease has not yet reached us

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Background and aims: According to recent epidemiological data, there is a shift in the epidemiology of hepatocellular carcinoma (HCC), with an increasing number of patients presenting with metabolic associated steatotic liver disease (MASLD) as an underlying cause. Our aim was to analyze the epidemiological evolution of HCC in our region from 2016 to 2023.

Method: We conducted an analysis of the prospective registry of patients from the specialized clinic at our center, which serves a population of 900,000 inhabitants. We defined the etiologies hierarchically: hepatitis C virus > alcohol > hepatitis B virus (and hepatitis D) > MASLD > others. We defined the degree of liver dysfunction based on the presence or absence of esophageal varices and the presence or absence of decompensations of cirrhosis prior to the diagnosis of HCC. To analyze the epidemiological changes through the time, we divided the 8-year study period in two ways: grouped into four-year intervals (2016-2019 vs. 2020-2023) and by comparing the extreme biennia (2016-2017 vs. 2022-2023).

Results: From January 14th, 2016 to December 29th, 2023, 940 patients with primary liver tumors have been included, of which 894 are HCC: 86% men, mean age 67 years, 271 HCV, 442 ALD, 20 VHB, 4 VHB + VHD, 54 MASLD, 103 others. 40.3% did not have esophageal varices nor had they presented prior decompensation of cirrhosis, 23.6% had esophageal varices without previous decompensation and 36.1% had presented some decompensation (75% ascites, 16.1% variceal bleeding, 8.9% encephalopathy). 69.8% were Child A and 53.2% diagnosed in BCLC-0-A stages.
At metabolic level, 33% had obesity, 39.1% had type 2 diabetes mellitus, 53.1% had arterial hypertension and 21.4% had dyslipidemia. 12.4% were evaluated for liver transplantation and 38.4% received loco-regional treatment. Median follow-up was 19 months and median overall survival was 29 months (IQR 9-102). There were no statistically significant differences in the etiological distribution or in the degree of liver dysfunction comparing both four-year periods or comparing the extreme two-year periods. We found differences in the prevalence of arterial hypertension (p=0.032), but not in obesity, diabetes or dyslipidemia. At the end of follow-up (Oct 1st, 2024), 521 patients (58.3%) had died, 68.1% due to liver failure/tumor progression.

Conclusion: In our region alcohol continues to be the main cause of HCC. We have not observed epidemiological changes in the last 8 years in terms of MASLD increase or less liver dysfunction at the moment of presentation of HCC.

POSTER ABSTRACT PRESENTATIONS

Nurses & AHPs

PO7-11-YI

Improvement of quality of care and patient satisfaction for patients with hepatocellular carcinoma via implementation of a specialist nurse – interim analysis of the HCC-Care Nurse pilot study

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Background and Aims: Implementing a hepatocellular carcinoma care nurse (HCCCN) has already been shown to be beneficial, cost-effective, and to reduce adverse events in patients with HCC treated with sorafenib. While the number of patients diagnosed with HCC is increasing, care nursing is not broadly available in Austria. The aims of this study are to standardise patient care by implementing an HCCCN in daily clinical practice and to evaluate this implementation on quality of life (QoL), incidence of emergency admissions as well as length of hospitalisation, occurrence and severity of adverse events and time on systemic treatment in patients with HCC.

Method: All consecutive patients with HCC treated with systemic therapy at the Medical University of Vienna will be included in this single centre, prospective pilot-study. Patients will be 2:1 randomised into the intervention arm (group A with HCCCN n=40) and the control arm (group B without HCCCN n=20). In addition to QoL questionnaires, a self-developed patient satisfaction questionnaire will be handed out to the patients. At each visit, patients will fill out the questionnaires and be seen by the treating physician, as well as by the HCCCN for the intervention arm. Additionally, in the intervention arm, telephone visits will be performed by the HCCCN every two weeks. Here, we report results of an interim analysis.

Results: To date, 12 patients have been enrolled (8 in group A, 4 in group B), with 9 being male. All were diagnosed with BCLC stage C, and 7 were classified as Child-Pugh grade A. Six patients received immunotherapy, while six were treated with tyrosine-kinase-inhibitors. Across both groups, patients had a median of two in-person visits (range: 0-7) over a median follow-up of 4 months (range: 2.5-5.3). Additionally, the intervention group received a median of one telephone visit (range: 0-7). A total of 34 patient satisfaction questionnaires were analysed. Initially, patients in group A showed uncertainty regarding long waiting times but later disagreed strongly, whereas group B reported worsening experiences over time. Both groups were initially unsure about reaching healthcare staff if needed. However, group A showed improved agreement over time, while group B declined. In terms of education on health protection and illness prevention, group A's responses improved from agreement to strong agreement, while group B remained uncertain.

Conclusion: The implementation of HCCCN is a viable proposition and may improve the QoL of patients with HCC. Recruitment in this study continues.

POSTER ABSTRACT PRESENTATIONS

Public Health

PO2-23
CRISPR/Cas9-Mediated BIRC5 Knockout Suppresses Proliferation and Migration while Promoting Apoptosis

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Background and aims: Hepatocellular carcinoma (HCC) is one of the most lethal cancers worldwide. There are still challenges for HCC treatments, especially high resistance of the cancer cells to chemotherapy and/or target therapy. In this study, bioinformatics analyses were conducted to identify the hub genes associated with HCC. BIRC5 gene was a top candidate target gene for knocked out using CRISPR-Cas9 system in HepG2 cell lines to evaluate its effect on tumor proliferation, migration and apoptosis.

Methods: BIRC5 gene specific single guide RNA (sgRNA) with CRISPR/Cas9 system was designed and cloned in pCAG-eCas9 CRISPR vector. The recombinant vectors with sgRNA and empty vector (negative control) were transfected into HepG2 cell culture by lipofectamine transfecting agent. The expression of survivin was investigated in transfected cell culture by real time PCR and Western blot. Tumor proliferation and apoptosis were measured using MTT and apoptosis assay, while migration and cell cycle were screened using wound healing and cell cycle assay, respectively.

Results: Our results demonstrated that BIRC5 gene knockout in HepG2 cell lines significantly reduced mRNA survivin level which subsequently reduced cell proliferation and migration along with inducing apoptosis compared to negative control.

Conclusion: The study provides evidence that CRISPR/Cas9-mediated BIRC5 knockout suppresses HCC cell proliferation and migration.

PO3-13-YI
Machine learning for early detection of cholangiocarcinoma in a population-level cohort

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Background and aims: Hepatobiliary cancers, especially hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) are highly fatal malignancies. Both are frequently diagnosed in advanced disease stages, limiting prognosis. We have previously reported a highly accurate machine learning algorithm to pre-screen patients with high risk of HCC development. We integrated demographic information, lifestyle and serum parameters to stratify patients at risk of HCC, outperforming all state-of-the-art risk scores in two population-sized cohorts with almost 1 million participants. Here, we aimed to build a risk stratification tool for CCA, given the complete lack of risk scores except for patients with primary sclerosing cholangitis.

Method: We trained machine learning models on multimodal UK biobank (UKB) data from > 400.000 participants. UKB is a population-based cohort with rich phenotypic characterization including lifestyle measurements, physical measurements as well as genomics (n~500k each) and metabolomics data (n=250k), linkage to health records and death registers, with 20 years of follow up. We trained random-forest-classifiers in a five-fold cross-validation on participants from England, with validation on participants from Scotland and Wales. Given the heterogeneity of CCA cases (n=668), additional models were trained on solely intrahepatic (iCCA, n=428) vs extrahepatic CCA cases (eCCA, n=113). Demographic variables, health records, blood parameters, genomics (18 single nucleotide polymorphisms associated with CCA) and metabolomics were hereby incrementally enriched to reflect clinically relevant scenarios without sophisticated omics techniques compared to omniscient perspectives.

Results: Our models achieved AUROCs of 0.7 in the validation set, with no additional benefit of addition of genomics or metabolomics over a model consisting of demographic variables, EHR and blood parameters. The iCCA-only and eCCA-only models achieved AUROC of 0.7 and 0.66, respectively. Interpretability analysis highlighted age as the most relevant feature, followed by ICD-codes like “complication of cirrhosis”, “obstruction of bile duct”, and blood parameters, e.g. “HbA1c”, “Gamma-GT”, “IGF-1”, “AP”, but also lifestyle factors such as “Pack years”.

Conclusion: Our work presents the first-ever risk score for CCA in a general population by leveraging machine learning. Our model, for which we provide all code and weights, can hereby serve as baseline for further prediction efforts for early detection of CCA in the general population.

PO5-06
Changes in incidence of primary liver cancer (hepatocellular carcinoma and intrahepatic cholangiocarcinoma) in Scotland between 2000 and 2019

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Background and aims: The incidence of primary liver cancer (PLC) has been reported to be increasing in many European countries in recent decades. Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA) are the most common PLC subtypes. We aimed to report recent trends in PLC incidence in Scotland prior to the COVID-19 pandemic.

Method: Individual-level data were accessed between 2000 and 2019 from the Scottish Cancer Registry, a nationwide, population-based registry. PLC patients were defined as ICD-O-3 C22 with all morphological codes. HCC patients were defined as ICD-O-3 C22 with 8170-8175, 8180 morphological codes and ICD-O-3 C22.0 with 8010, 8140 morphological codes. iCCA patients were defined as ICD-O-3 C22 with 8160-8161 morphological codes and ICD-O-3 C22.1 with 8010, 8140 morphological codes. European Age-Standardised Incidence Rate (EASIR) was calculated. Joinpoint regression analysis was applied to examine the incidence trend.

Results: PLC, HCC and iCCA EASIR were calculated for each year in 2000-2019 in Scotland. PLC incidence for all persons increased between 2000 [n = 263, EASIR = 6.2 (5.4, 6.9) per 100 000] and 2014 [n = 627, EASIR = 12.4 (11.4, 13.4) per 100 000]. It did not change from 2014 to 2019 [n = 669, EASIR = 12.3 (11.4, 13.3) per 100 000]. HCC incidence for all persons increased between 2000 [n = 117, EASIR = 2.7 (2.2, 3.2) per 100 000] and 2013 [n = 415, EASIR = 8.3 (7.5, 9.1) per 100 000]. It did not change from 2013 to 2019 [n = 429, EASIR = 7.8 (7.1, 8.6) per 100 000]. iCCA incidence for all persons increased between 2000 [n = 106, EASIR = 2.5 (2.0, 2.9) per 100 000] and 2015 [n = 218, EASIR = 4.3 (3.7, 4.8) per 100 000]. It did not change from 2015 to 2019 [n = 198, EASIR = 3.7 (3.2, 4.2) per 100 000]. Secondly, to describe how PLC patient characteristics have changed over time, the end of 2014 was selected as the cut-off point based on Joinpoint regression analysis. Among PLC subtypes, the proportion of HCC (compared to iCCA and others) increased from 2000-2014 to 2015-2019 ($p < 0.05$).

Conclusion: PLC incidence increased in 2000-2014 in Scotland but did not increase in 2015-2019. The proportion of HCC among PLC increased during the same period. The increase in PLC incidence was mostly driven by the increase in HCC incidence. These trends have implications for primary prevention and specialist care.

PO6-17
Economic evaluation of the hepatocellular carcinoma early detection and treatment presidential initiative in Egypt

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Background and aims: Hepatocellular Carcinoma (HCC) is the most prevalent form of primary liver cancer and represents a significant global health challenge. It ranks as the third leading cause of cancer-related deaths worldwide, with over 780,000 fatalities reported in 2018. The increasing incidence of HCC, especially in areas with high rates of hepatitis B and C infections, alcohol abuse, and other liver diseases, highlights the urgent need for effective prevention and treatment strategies. Notably, liver disease associated with Hepatitis C is the leading cause of HCC, accounting for approximately 50% of cases. Building on the success of the Hepatitis C Virus (HCV) Eradication Initiative in Egypt, an early detection and management of HCC initiative was launched to address this critical health issue. This study aims to evaluate the long-term health and economic benefits of the HCC Presidential Health Initiative in Egypt.

Method: A decision tree model compares the initiative versus no-initiative scenarios, followed by a cost-benefit analysis (CBA) using a Markov model. The model accounts for initiative costs, HCC management costs, life years, QALYs, and productivity loss due to premature death. It includes different stages of HCC according to the Barcelona Clinic Liver Cancer (BCLC) staging system: Stage 0-A, Stage B, Stage C, and Stage D. Improved access to systematic treatments was achieved to HCC patients in this initiative. The evaluation uses initiative logs and a comprehensive literature review, including both direct and indirect costs associated with both scenarios. The analysis considers a lifelong time horizon, with a 3.5% discount rate applied to costs and outcomes.

Results: 97,194 patients were screened resulting in 2,100 patients diagnosed with HCC. The initiative resulted in total costs of EGP 514,954,007 (USD 10,661,573) compared to the no-initiative scenario of EGP 651,792,299 (USD 13,494,663), saving 3,872 life years and yielding extra 1,505 QALYs versus no initiative scenario. The ROI for the initiative is 26.6%, with economic savings of more than 136.5 million EGP (USD 2.83 million).

Conclusion: The HCC Presidential Health Initiative demonstrates significant clinical and economic benefits, including improved health outcomes and cost savings. This study highlights the significant health and economic benefits of early HCC detection and treatment. The findings are crucial for policymakers, healthcare providers, and researchers, emphasizing the importance of sustained and expanded screening initiatives. These findings support the continued implementation and potential expansion of the initiative to enhance HCC prevention and treatment efforts in Egypt.

PO7-12-YI
The good and the bad for patients with HCC in the pre and post COVID pandemic years

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Background and aims: In 2022 we reported an alarming fall in patients with hepatocellular carcinoma (HCC) referred to our Newcastle-upon-Tyne NHS foundation Trust HPB multidisciplinary team (MDT), in the in the year immediately following the pandemic. Furthermore, we documented a fall in cases detected by surveillance, with a proportional increase in cases presenting symptomatically [Geh et al. BMJ Open Gastroenterology, 2022]. We have assessed the ongoing impact.

Method: We identified patients with a new diagnosis of HCC referred to our HPB MDT, comparing 12 months pre-pandemic (March 2019-Feb 2020), pandemic (March 2020–Feb 2021) and post pandemic (March 2021–Feb 2022, March 2022-Feb 2023). Mode of presentation, stage, treatments and survival over the 4 years were compared, alongside a historical MDT cohort (632 patients, 2004 and 2010) [Dyson et al, J Hepatol, 2014].

Results: Annual MDT referrals started to recover post pandemic, (159 and 170 patients, compared to pre-pandemic 189 and pandemic 120; total n=638). Cases detected by surveillance also recovered, although incidental cases (largely well man check, primary care) have not and symptomatic cases have risen (Surveillance/incidental/symptomatic 2019-2020 34%/42%/24% vs 2020-2021 26%/33%/40% vs 2022-2023 33%/23%/44%; p<0.001, Pearson Chi Square), with the majority diagnosed with BCLC-C or BCLC-D HCC. This is similar to our historical cohort 2004-2010, n=632 (25%/32%/43%). While survival was markedly better in patients detected by surveillance or incidentally, median months survival in symptomatic patients has improved over the 4 years (5.1, 6.5, 6.9, 16.5, p=0.005, n=231). Notably, compared to our historical cohort, overall survival has significantly improved (2004-2010/2019-2020/2020-2021/2021-2022/2022-2023 – 10.7/15.5/15.9/16.6/27.1, p<0.001), associated with increased treatment options for those with advanced disease (selective internal radiotherapy, atezo/bev, resection), as well as enhanced supportive care.

Conclusion: This audit highlights, consequent to the impact of the pandemic and increasing pressures on our NHS, only one third of our HCC patients are detected by surveillance, fewer are detected incidentally and nearly half still present symptomatically. Despite this appalling lack of improved early detection in over 20 years, it is evident that regardless of stage of disease or presence of symptoms, there are more treatment options and the prognosis of these patients has improved.

PO7-16
Prognosis of HCC in French Guiana and the French West Indies Compared to a Tertiary Center in the Île-de-France Region

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Background and aims: Limited data exist on the prognosis of hepatocellular carcinoma (HCC) in French Guiana (GUY) and the French West Indies (GUA). This study aimed to describe HCC characteristics and outcomes in GUY and GUA compared to a tertiary center in Île-de-France (IDF).

Method: Patients presented in multidisciplinary tumor boards from 2013–2023 in GUY, GUA, and IDF were retrospectively included, with data at initial diagnosis. Overall survival (OS) was analyzed using Kaplan-Meier and Cox proportional hazards models.

Results: A total of 1,114 patients were included (GUY: 45; GUA: 71; IDF: 998). HBV prevalence was higher in GUY (42%) and GUA (38%) vs. IDF (16%) (p<0.001). Alcohol-related HCC was more frequent in GUA (55%) and IDF (50%) compared to GUY (31%) (p=0.03). MASLD was more common in GUY (24%) and IDF (29%) than GUA (9%) (p<0.001). Cirrhosis rates were lower in GUY (76%) vs. GUA (100%) and IDF (85%) (p<0.001), with only 25% classified as Child-Pugh A in GUY compared to 57% in GUA and 70% in IDF (p<0.001). GUY had higher MELD scores (median 15) than GUA (10) and IDF (9) (p<0.001). Advanced-stage diagnosis was more common in GUY (77% outside Milan criteria) than in GUA (60%) and IDF (49%) (p<0.001). BCLC-D classification at diagnosis was seen in 40% of GUY patients vs. 17% in GUA and 5% in IDF (p<0.001). Only 11% in GUY received curative treatment vs. 28% in GUA and 50% in IDF; 53% in GUY had no treatment (p<0.001). Median OS was 2.5 months in GUY, 34.5 months in GUA, and 22.7 months in IDF (p<0.001). Multivariate analysis showed BCLC-C (HR=1.93) and BCLC-D (HR=4.81) were linked to higher mortality, while care in GUA (HR=0.28) and curative treatment (HR=0.37) improved survival. For BCLC-0/A patients, 43% in GUY received curative treatment vs. 77% in GUA and 87% in IDF (p=0.004). OS for BCLC-0/A was similar (~85% 12-month survival) across centers (p=0.14). Higher age (HR=1.03), MELD score (HR=1.11), and infiltrative HCC (HR=1.85) were associated with mortality. For BCLC-B patients, GUA primarily used systemic treatments (65%), while IDF favored radiologic methods (p<0.001). Median OS was similar (19.2 months in IDF, 16.2 months in GUA, p=0.94). AFP ≤ 2 (HR=0.47) and curative treatment (HR=0.49) were linked to better survival.

Conclusion: HCC prognosis in GUY remains poorer compared to GUA and IDF, with late-stage diagnoses limiting curative options. Improved healthcare access and preventive strategies are crucial in GUY.

PO7-22-YI
Cholangiocarcinoma in Latin America: A Multicenter Observational Study Alerts on Ethnic Disparities in Tumor Presentation and Outcomes

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Background and aims: Cholangiocarcinoma (CCA) represents a global health challenge, with rising incidence and mortality rates. This study aimed to elucidate the clinical course and practices of CCA in Latin America.

Method: This observational cohort study investigated individuals diagnosed with CCA between 2010 and 2023 at five referral centers across Latin America. Demographic, biochemical, and clinical data were analyzed.

Results: A total of 309 patients were enrolled, demonstrating a balanced distribution of CCA subtypes (intrahepatic, perihilar, and distal), with Hispanics and Caucasians as the predominant ethnic groups, followed by Africans. Major risk factors identified included age, diabetes, obesity, MASLD, bile duct stones, and cholecystitis. Disparities in overweight/obesity prevalence were noted among CCA subtypes and ethnicities, with higher rates in extrahepatic CCA and among Hispanics and Caucasians. At diagnosis, 72% of patients had ECOG-PS scores of 0-1, with disease presentations ranging from localized (47%) to locally advanced (19%) and metastatic (34%). Patients who did not receive any anti-cancer therapy exhibited a median survival of 2.3 months. Survival rates significantly improved across treatment modalities, with surgery yielding the longest (34 months), followed by chemotherapy (8 months). Notably, Africans presented with worse ECOG-PS scores and more advanced disease, while Hispanics were less frequently treated with chemotherapy for advanced disease, contributing to lower survival rates (8.3 and 6 months, respectively) compared to Caucasians (12.6 months).

Conclusion: The high prevalence of late-stage CCA diagnosis in Latin America, particularly among individuals of African ethnicity, coupled with a significant proportion of Hispanic patients not receiving chemotherapy, underscores the dismal prognosis for these patients. These findings reveal structural challenges in cancer screening and healthcare access among diverse ethnic backgrounds and lower

socioeconomic statuses in the region. Urgent measures are needed, including the identification of preventable risk factors, raising awareness among high-risk populations, and establishing equitable health coverage to address these disparities.

PO7-23-YI
Ethnic and racial disparities in hepatocellular carcinoma: “real-world” data from a Southern California clinic show worse outcomes in Hispanics/Latinos

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Background and aims: Hepatocellular Carcinoma (HCC) outcomes in the USA may be influenced by race/ethnicity resulting in differences from obesity, diabetes (T2DM), MASLD, and socioeconomic factors. We aim to discern disparities associated with HCC in a diverse population at a private Hepatology practice in Los Angeles County, California, USA.

Method: Retrospective study within 6m of initial HCC evaluation at our clinic from 2018-2023, diagnosed by standard MRI/CT criteria. Liver disease etiology, presence of cirrhosis, clinical characteristics, and socioeconomic factors including Area Deprivation Index (ADI) were stratified by ethnicity/race and compared by univariate and multivariate analyses.

Results: Among 245 patients, 45.9% were Hispanic/Latino (HL) (76.6% Other and 23.3% White), 54.1% Non-Hispanic/Latino (NHL) (54.0% Asian, 7.3% Black, 27.4% White, 11.3% Other). Median age was 71y (27-88). 73.5% were male. 89.0% had cirrhosis from alcohol-associated liver disease (ALD) (13.9%), HBV (17.6%), HCV (29.4%) and MASLD (33.9%). 55.9% were within Milan criteria, 72.2% within UCSF criteria. 19.8% had an AFP ≥ 500. 27.3% underwent curative locoregional therapies (CLRT:RFA/surgical resection), 40.8% non-curative locoregional therapies (NCLRT:TACE/TARE), 14.7% systemic therapy, 15.1% untreated, and 30.2% died. HLs had higher T2DM prevalence (54.2% vs 29.4%, p = 0.0001), BMIs [28.0(18.3-49.0) vs 25.9(14.3-50.0), p = 0.0055], MELD [9.5(6-29) vs 8.0(6-22), p = 0.0444]; and ADI [15(2-53) vs 12(1-84), p = 0.0014), notably between Other-HL and Asians [15(2-34) vs 12(1-42), p = 0.013]. HLs had cirrhosis mostly due to MASLD (53.3% vs 34.8%, p < 0.0001) and ALD (17.8% vs 10.3%, p<0.0001). MASLD patients had the highest ADI [15(2-53), p = 0.0300]; 70.4% of MASLD were HL. Most with HBV were Asian (93.0%, p < 0.001). Non-cirrhotic HCC was mostly HBV and MASLD (32%, 4.8%, p < 0.0001). Majority receiving CLRT were Asian (42.2%, p =0.05), with 25.4% undergoing surgical resections (p = 0.0002). Systemic therapy recipients were majority HL (67.7%, p = 0.0030), had MASLD (44.4%), AFP ≥ 500 (50.0%, p < 0.0001), and died (58.3%, p < 0.0001). 33.1% had cirrhosis diagnosed concomitant to HCC diagnosis; 41% were diagnosed through surveillance. Variables independently associated with systemic therapy and death were White-HL, T2DM, MELD, Milan or UCSF criteria.

Conclusion: HLs are the most socially disadvantaged, exhibit higher incidence of cirrhosis due to MASLD and ALD, non-cirrhotic HCC (second to Asians with HBV), higher BMIs, and diabetes prevalence. HLs present with more advanced disease leading to the greatest need for systemic therapy. HBV and surgical resection rates were highest in Asians.

PO8-18
KAIGI HCC – A web-based platform for discussion of hepatocellular carcinoma (HCC) treatment opportunities

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Background and aims: Liver cancer is the fifth most common cancer and the third most common cause of cancer death worldwide. The number of new cases could increase by >55% by 2040. Patients with HCC are classified into five stages based on specific variables (BCLC score). For Germany, the diagnostic and treatment concepts are summarized in three complementary clinical guidelines (HCC 2024, MAFLD 2024, liver transplantation 2023). However, the diagnosis and treatment of HCC is complex. In addition to the simultaneous treatment of at least three diseases (underlying liver disease, liver cirrhosis/post-LTx and HCC), the current therapy algorithms/innovations represent major clinical challenges. This results in the need for treatment in an interdisciplinary/intersectoral team and in the desire for an easy-to-use exchange platform for practical implementation in everyday clinical practice.

Method: KAIGI HCC is a multidisciplinary, intersectoral, regional HCC exchange platform developed as part of a medical hackathon and designed by HCC practitioners. The platform is login-secured and offers the opportunity to discuss topics related to HCC with specialists. The focus here is on providing patient care in accordance with guidelines and includes the entire spectrum of prevention, screening, diagnosis, treatment and follow-up care in a network. Improved communication between doctors allows treatment to begin earlier, resulting in more potential treatment options.

Results: The exchange on KAIGI HCC is possible by discussion either in a public forum or private groups. Additionally the platform provides the possibility to share clinical cases and overview material. Lastly video calls can be held with HCC/transplant experts and appointments can be planned. This enables faster and more straightforward coordination between referrers and experts. An initial user survey yielded consistently positive feedback.

Conclusion: KAIGI HCC offers guideline-based support for HCC therapy decisions for the current therapy migration concept. KAIGI HCC can be individually adapted to regional HCC therapy structures and supports the concept of intersectoral care.

PO9-01-YI
Hepatitis delta virus (HDV) replication through HBV integrants in HCC recurrence after liver transplantation

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Background and aims: Hepatitis D Virus (HDV) is a defective RNA virus and requires the helper function of HBV for viral assembly and in vivo transmission. It is a highly pathogenic virus that causes the least common but most severe and rapidly progressive chronic hepatitis, leading to cirrhosis in about 80% of the cases within 10 years. HDV cirrhosis may be a stable disease for many years. Still, a high proportion of patients eventually die of hepatic decompensation or hepatocellular carcinoma (HCC) unless they undergo liver transplantation (LT).

Method: A PWID man, HCV/HBV-HDV/HIV-infected, underwent LT for HCC in 2012 at the age of 52 years. HCC tissue showed high HDV-RNA (88,400 copies/cell), low total HBV-DNA (0.00001 c/c), and HBVcccDNA0.00008 c/c), without detectable HBV-RNA. High-throughput HBV integration sequencing (HBIS) identified 657 HBV integration sites.HBx gene sequences predominantly represented HBV integrants. After LT, Tacrolimus, Bictegravir/Emtricitabine/TAF, and anti-HBs immunoglobulin were administered, yielding HBsAg, HDV-RNA, and HCV-RNA negativity.

Results: In 2018, HBsAg reversion was observed with undetectable HBV-DNA and HDV-RNA >19,000 c/ml.In 2019, HDV-related hepatitis occurred. Intrahepatic HBcAg, HBsAg, HBV DNA, HBVcccDNA, and HBV-RNA were undetectable. HDV RNA concentrations were very high in the liver (3,920,000 c/c) but low in the serum (214 IU/mL). CT scan (CTs) suspected an isolated HCC recurrence in the left adrenal gland, confirmed by adrenalectomy. Real-time PCR in the tumor from the adrenal gland revealed high levels of HDV RNA (5.5 c/c) but low levels of HBV DNA (0.00009 c/c) and HBVcccDNA (0.00001 c/c). HBV RNA was undetectable. HBIS identified 3497 HBV integrations, most of which included HBs gene sequences. After adrenalectomy, HBsAg and HDV-RNA became undetectable. Anti-HBs immunoglobulin was continued with Everolimus. In 2021, CTs showed two HCC nodules in the liver and one in the right adrenal gland. TACE was performed, and TKI therapy was started. In 2023, new HDV hepatitis occurred, with HDV-RNA>3,631,360 UI/ml and HBV-DNA <10UI/ml. For the progression of HCC, RFA on the right adrenal gland was performed, and Bulevirtide was started. After 3 months, HDV-RNA was 48,638 c/ml, and transaminases were normal.

Conclusion: This case demonstrates HDV replication in extrahepatic HCC recurrence, despite low levels of HBVcccDNA. The decreased HDV RNA levels after RFA and BLV therapy suggest that HCC metastases may serve as HBsAg production sites following HBV integration.

PO9-07-YI
Democratizing access to healthcare through Point of Care Testing (POCT)

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Background and aims: Hepatitis C Virus (HCV) infection is a prevalent global community concern. Recently developed Direct Acting Antiviral Drugs offer significant opportunity for not only cure of the estimated fifty million individuals globally with HCV, but elimination of HCV as a global public health threat, respecting and attaining World Health Organization (WHO) goals of elimination as a public health threat by 2030.
A major challenge in reducing the burden of care on health systems, as asymptomatic HCV infection progresses to chronic conditions, is scaling up standardized screening and testing. This is especially critical for People Who Inject Drugs (PWID), a high-risk priority population for HCV infection. Due to the illegal nature of injection drug use, PWID are a highly stigmatized and vulnerable group, making it extremely difficult to reach them for diagnosis and treatment.
A key resource to facilitate HCV treatment of PWID involves the peer workforce of those with lived and living experience of injecting who, in this definition, have the advantage of being understood and accepted as frontline workers by PWID, and are a key resource to population wide HCV treatment enabling decentralized community-based practice, care of community by community.
The intended outcome of researching "*How can we improve efficacy of an informally qualified community of testers and their structured inclusion in established systems through leveraging EDT and associated technologies?"* is to contribute to formalization and recognition of skill for peers allowing supported relationships with those seeking health through navigation of treatment and continuation of care. Thereby supporting engagement in enhancing predominately under resourced, under financed and over committed primary and secondary medical care systems.

Method: Further extrapolation of the main question formed 33 survey questions, disseminated electronically for anonymized completion. Secondary research and literature review drew from 107 reference samples.

Results: Respondents demonstrated overwhelming support for leveraging Emergent Disruptive Technologies (EDT) and associated technologies to improve efficacy of an informally qualified community of testers and their structured inclusion in established systems.

Conclusion: By leveraging of EDT and associated technologies to formalize qualification and provide a systemized supported framework for facilitating HCV care navigation by peers; we can contribute to financial, health and community benefits with improved destigmatized health care access and lessened burdens on existing health systems.
Note: Developing solutions based on these research findings, are available at https://HealthNetworkCollective.com for expected free license distribution 2025.

PO9-13
Mongolian liver cancer ethiologic description analysis

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Background and aims: Mongolia has the world's highest prevalence and mortality of hepatocellular carcinoma (HCC). Despite this, the etiology of HCC has not been thoroughly described. The aim of this study was to delineate the underlying causes of liver cancer among participants enrolled in the DETECT-HCC study.

Method: We analyzed data from 364 HCC participants in the DETECT-HCC study, enrolled between Sep 15, 2023, and Sep 23, 2024, through voluntary recruitment.
HCC diagnosis was confirmed with CE-MRI. Hepatitis viral infection status in each participant was assessed using qHBsAg and qAnti-HCV tests (by HISCL5000 analyzer). Additionally, HDV infection status was evaluated in all qHBsAg+ individuals using an ELISA test.
HBV-DNA and HCV-RNA loads was conducted using the Cepheid(GeneXpert), while HDV-RNA analysis was carried out using the PCR (Agilent).
Whether or not the participant has cirrhosis was evaluated by both clinically and radiologically.

Results: Total 364 cases (average age= 61.8 years; IQR=5 range between 31 to 91) which are 208 male (average age= 59.7; IQR= 12 range: 38-88) and 156 female (average age= 64.4; IQR= 12 range: 31-91) participants. Among these, 128 (35.16%%) HCV infection (average 67.5 years), 174 (47.8%) HBV,HDV dual-infection (average 57.9 years), 25 (6.9%) HBV infection(average 60.4 years) and 35 (9.6%) both qHBsAg and qAnti-HCV negative cases were identified. Also, there was 1 case (0.3%) each of HBV,HCV dual, and HBV, HDV, HCV triple-infection, respectively. In the anti-HCV positive group, gender ratio was 51 males, 77 females, while, in the HBsAg positive group was 132 males, 69 females. In the qAnti-HCV positive group, 62 individuals had detectable HCV-RNA, while 66 had no detectable HCV-RNA. Additionally, 19 cases in the qHBsAg and qAnti-HCV negative groups were attributed to alcohol-related liver disease.
58.1% of HCV-RNA detected group, 33.3% of HCV-RNA not detected group, and 58.6% of HBV,HDV dual-infection group were diagnosed with liver cirrhosis.
The total cases, classified according to the BCLC staging system, is as follows: BCLC-0, 25; BCLC-A, 85; BCLC-B, 179; BCLC-C, 65; BCLC-D, 10 cases.

Conclusion: Based on this analysis, the main causes of HCC in Mongolia are viral hepatitis. Also HBV, HDV dual-infection accounted for 47.8% and 17% of all HCC cases had detectable HCV-RNA, indicating a need for further investigation and improvement in management. Additionally, 66.6% of HCV-RNA not detected group were diagnosed with HCC even without Liver Cirrhosis. Also HBV, HDV dual-infected subjects were significantly younger than other groups.

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