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Abstracts

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Disclosure Statement

The abstracts included in this supplement were reviewed and selected by Scientific Committee responsible for Abstract Selection. The committee has no conflicts of interest in connection with the congress and the selection of abstracts.

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Lecture Abstracts

Pre-congress Workshop–Topic 1

PWT-1

The adjuvant therapies after curative treatment of HCC

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Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths worldwide. Most cases of HCC in Asia are related to hepatitis B, a virus prevalent in the region. Liver transplantation offers the best survival for patients suffering from HCC. However, due to limitations of the shortage of liver grafts, and the fact that not all HCC patients are eligible for transplantation, hepatectomy remains an important curative measure and can achieve a satisfactory survival outcome with a 5-year survival rate of 72.8%. The ultimate goal of a hepatectomy is to achieve negative resection margins, which have been shown to be an important prognostic predictor. The risk of recurrence of HCC remains high due to nature of the disease. Various methods have been proposed to reduce the recurrent rate by administration of adjuvant therapy. These includes transarterial chemoembolization, immunotherapy, targeted therapy such as sorafenib, and interferon therapy, antiviral therapy, Hepatic artery infusion therapy (HAIT) etc. In this session, the efficacy and evidence of these therapies will be discussed. **Keywords:** hepatocellular carcinoma; Asia; adjuvant therapy; efficacy; evidence.

Presidential Lecture

PL-1

Therapeutic strategy to improve oncologic outcome of HCC in immuno-oncology era: Radiotherapy

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In the immune oncology era, the clinical efficacy of immune checkpoint inhibitors (ICIs) against most solid cancers is well known. In hepatocellular carcinoma, clinical outcome of ICI monotherapy has been unsatisfactory, which led to testing combination therapy of various agents involving ICIs and molecular targeting agents. Then, recent success of combination therapy, atezolizumab and bevacizumab, opened a new immune oncology (IO) era in HCC treatment. While this combination therapy stands as a standard of care for advanced HCC, we still need therapeutic strategy, which can further improve oncologic outcome. In this context, radiotherapy (RT) seems an attractive modality to combine with IO agents. First, radiation can enhance IO effect not only by remodeling tumors from immune-cold to immune-hot status but also by decreasing tumor burden, which seem significant factors in escalating IO effect. Second, RT can do substantial role in downstaging/downsizing through multimodal treatment, which facilitates conversion to resectable/transplantable status. Finally, RT can do a significant role in metastasis-directed therapy by ablating metastatic lesions in addition to systemic agents, which can suppress progression to full metastasis and prolong overall survival in oligometastatic HCC. In conclusion, radiotherapy could be a useful therapeutic strategy to improve oncologic outcome of HCC in immuno-oncology era. **Keywords:** hepatocellular carcinoma; combination therapy; radiotherapy; immune oncology.

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State-of-the-Art Lecture-1

SL1-1
Immunotherapies for hepatocellularcarcinoma

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Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death and its incidence is increasing globally. Around 50–60% of patients with HCC receive systemic therapies, traditionally sorafenib or lenvatinib in the first line and regorafenib, cabozantinib or ramucirumab in the second line. In the past 5 years, immune-checkpoint inhibitors have revolutionized the management of HCC. The combination of atezolizumab and bevacizumab has been shown to improve overall survival (OS) and quality of life relative to sorafenib, thus representing the dawn of a new era in the management of this neoplasm. More recently, the combination durvalumab and tremelimumab also showed improvement over sorafenib in terms of OS. In addition, nivolumab with ipilimumab and pembrolizumab have received FDA Accelerated Approval in the second-line setting based on early efficacy data. More than ten phase III studies are assessing the role of immune-based regimens in the adjuvant setting, intermediate stage in combination with chemoembolization and advanced stages of the disease. Despite these major advances, the molecular traits governing immune responses and evasion remain unclear, and there are no available biomarkers to segregate for response or better outcome. Recent data pointed towards a distinct etiology-dependent immune features that need to be confirmed, particularly for the subpopulation of NASh-HCC. Overall, immune based therapies have represented a substantial improvement of the management of HCC patients, and further advancements on the understanding of the mechanism of response and resistance are expected to be translated in clinical benefits. **Keywords:** hepatocellular carcinoma; immunotherapy; immune-checkpoint inhibitor.

Session 1: HCC Surveillance and early diagnosis

S1-1
HCC surveillance: Korean experience

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Hepatocellular carcinoma (HCC) is one of the most common malignancies with high cancer related annual death rates worldwide. As HCC is a silent killer, most are diagnosed at advanced stage because most patients are asymptomatic. Therefore, it is important to diagnose HCC at earlier stage which is at curable stage. The rationale for surveillance for cancer is to reduce disease-related mortality. The surveillance strategy was associated with a higher rate of detection of small HCC. Fortunately, HCC has well-established risk factors that allow the identification of an at-risk patient group. At present, a strategy of surveillance with ultrasonography (US) and AFP measurement every 6 months targeting risk group such as liver cirrhosis or chronic liver disease significantly reduced HCC-related mortality. The sensitivity of detecting early stage HCC in high-risk patients is reportedly approximately 60%. Based on Korean cancer registered data, 5 year survival rate of HCC has been remarkably improved within 25 years from 11.8% to 37.7%. One of key reason is active implementation of surveillance program for HCC by national cancer screening program and active campaign and education of the necessity of surveillance by KLCA including liver cancer day. As the performance of surveillance strategy is still suboptimal, it is important to improve accuracy, sensitivity and specificity. There are many studies in Korea to improve detection rate by applying more advanced imaging tool such as MRI or dynamic CT scan. However, not only accuracy but costs, and potential harms regarding these new radiological modalities need to be considered before the wide implementation of the alternative surveillance imaging strategies. At present, the recommendations of Korean practice guidelines for the surveillance of HCC are as follows; [Recommendations] 1. Surveillance for HCC should be performed in high-risk groups; patients with chronic hepatitis B (A1), chronic hepatitis C (B1), and liver cirrhosis (A1). 2. Surveillance test for HCC should be performed with 1051 KLCA and NCC Korea: 2018 Practice Guidelines for HCC kjronline.org https://doi.org/10.3348/kjr.2019.0140 liver US plus serum AFP measurement every 6 months (A1). 3. If liver US cannot be performed properly, liver dynamic CT or dynamic contrast-enhanced MRI can be performed as an alternative (C1). **Keywords:** hepatocellular carcinoma; surveillance strategy; Korea experience.

S1-2
aMAP: a novel risk score for HCC in chronic liver diseases during antiviral era

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The World Health Organization (WHO) set a goal of reducing hepatitis-related mortality by 65% by 2030. In the era of antiviral treatment, hepatocellular carcinoma (HCC) is the leading cause of death in patients with chronic viral hepatitis and the fourth most frequent cause of cancer-related death globally. Therefore, the key to achieve the goal is to reduce the mortality of viral hepatitis-associated HCC. Hepatitis B and C virus (HBV, HCV) infections are the leading causes of HCC development. Over the past decades, a number of HCC risk scores have been developed and validated to stratify the risk of HCC development, however, most of these scores assign heavy weighting to viral factors and perform only satisfactorily among populations with specific aetiologies and ethnicities, thus limiting their widespread promotion and application worldwide in current era of sustained viral suppression or clearance. Recently, our group developed and externally validated a simple, objective, and

accurate prognostic tool (called the aMAP score) comprising routinely laboratory parameters (albumin, bilirubin, and platelets) plus age and sex that could satisfactorily predict the risk of HCC development among over 17 thousand patients with viral or non-viral hepatitis from 11 global prospective studies. aMAP score had excellent discrimination and calibration in assessing the 5-year HCC risk among all the cohorts irrespective of aetiology and ethnicity and is a useful tool for improving early HCC detection. Recent advances in studying circulating cell-free DNA (cfDNA) brought the non-invasive detection of HCC to a higher level. Emerging evidence suggests different molecular signatures present on cfDNA, including DNA methylation patterns, copy number aberrations, etc., are related to cancer pathobiology and have been shown as a feasible biomarker in HCC, thus proposing several cfDNA-based models to detect cancer at an early stage. In 2021, HIFI model integrating four cfDNA features (5-hydroxymethylcytosines/base mismatch/fragmentation/nucleosome footprint) could detect HCC from cirrhosis, achieving 95% sensitivity at the specificity of 97%. Theoretically, cfDNA signature would show specific somatic mutations at the molecular stage of HCC, therefore, it is a potential biomarker in diagnosing early/very early HCC. In the future, it is necessary to develop more accurate models involving common risk factors and cfDNA signatures to further improve the diagnosis accuracy of early HCC. **Keywords:** hepatocellular carcinoma; risk score; chronic liver diseases; the aMAP score; biomarker.

S1-3

Cost-effectiveness of chronic hepatitis C screening and treatment

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Hepatitis C virus (HCV) infection is the second most common cause of chronic liver diseases in South Korea. The prevalence of HCV infection in South Korea ranges from 0.6% to 1.5%, and HCV incidence increases with age. The anti-HCV antibody test is widely used to screen HCV infections and is cheaper than the HCV RNA assay. Under-diagnosis is a major barrier to the elimination of HCV infection. Although there are several risk factors of HCV infection such as persons who inject drugs, individuals who have history of blood transfusion and hemodialysis, most patients with HCV infection have no identifiable risk factor. Thus, strategy of universal screening for HCV in adult population is suggested to eliminate virus. Here, I will present the cost-effectiveness of HCV screening, as well as methodologies used for evaluation. Recent studies have shown that HCV screening and treatment using direct-acting antivirals are cost-effective approaches. However, the optimal timing and frequency of HCV screening are still unclear. Further studies are required to enhance awareness for elimination of HCV infection. **Keywords:** hepatitis C virus infection; South Korea; screening; treatment; cost-effectiveness.

S1-4

Changing epidemiology of HCC in Asia

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Liver cancer is the seventh most common cancer and the fourth leading cause of cancer death. Hepatocellular carcinoma (HCC), the most common subtype, accounts for 80%. The incidence and mortality of HCC show large geographic disparities. Of the 661,000 new HCC cases worldwide in 2018, Asia contributed 485,700 (73.5%). Eastern Asia had the highest age-standardized incidence rate (ASIR) of 23.1 per 100,000 and 6.7 per 100,000 in men and women, followed by South-Eastern, South-Central and Western Asia. China bears the greatest burden, with 301,500 new cases. HCC is a complex disease with different etiologies. The major risk factors in Asian include Hepatitis B or C virus (HBV/HCV) infection, exposure to aflatoxin-contaminated food, alcohol consumption, diabetes and obesity. HBV infection accounts for over 60% of HCC cases in Asia, and 80% in China, whereas HCV shares 67% in Japan. Implementing HBV vaccination and HCV treatment programs shift HCC epidemiology from mainly caused by virus infection to by lifestyle factors and metabolic factors. Over the past two decades, the ASIR of HCC has decreased due to a decrease in virus infection in many Asian countries, including Japan, China, Thailand, and Singapore. Although the decrease has been observed in Japan since 1990 and decreased by 3.9% per year in China during 2006-2015, overall new cases are still increasing, mainly due to population growth, aging and changing risk factors. Given the Asia prevalence of HBV infection is still high, especially among the elderly, HCC will continue to be a major disease burden, and the number of new liver cancer cases in Asia is projected to be 1.6 times higher in 2040 than in 2020. HCC is a lethal disease, and the 5-year net survival was 5-30% during 2000-2014. The survival for liver cancer were generally stable during 1995-2014 but increased by more than 10% in Korea and Singapore. Given the strong association between early detection and HCC survival, enhancing surveillance and diagnostic performance are prioritized in high-risk populations, and improving risk stratification is also urgently needed. A substantial proportion of HCC mortality might be amenable to primary prevention such as HBV vaccination, adaption of a healthy lifestyle, coffee consumption and aspirin use. Strengthening efforts are still needed in HCC prevention and control. **Keywords:** hepatocellular carcinoma; epidemiology; change; Asia; China.

Session 2: Innovation in systemic treatment for HCC and iCCA

S2-1

Adjuvant therapy for resectable biliary tract cancer

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Background: Surgical resection is the only potentially curative treatment, although the risk of relapse remains extremely high. The reported 5-year overall survival is 30–50%, which remains poor prognosis. Surgical resection alone has limitations in improving treatment outcomes, and attempts have been made to develop adjuvant therapies. Several randomized controlled trials as compared to surgery alone have been conducted as postoperative adjuvant chemotherapy, such as mitomycin C+5-FU for resected pancreatic and biliary tract cancer, 5-FU+Folinic acid and gemcitabine for resected periampullary cancer (ESPAC-3), gemcitabine for resected extrahepatic cholangiocarcinoma (BCAT), and gemcitabine+oxaliplatin for resected biliary tract cancer (PRODIGE 12-ACCORD 18). However, no trials showed significantly prolonged relapse-free survival or overall survival in comparison to surgery alone and had not established to be s standard treatment. **Methods:** Two phase III trials with orally administered fluoropyrimidine agents have reported favorable results in patients with resected biliary tract cancer. The first one is the BILCAP trial comparing capecitabine with surgery alone. **Results:** The primary endpoint of overall survival in Intent to treat analysis were median 6.4 months in the surgery alone group and 51.1 months in the capecitabine group (HR 0.81; 95% CI: 0.63-1.04, p=0.097), and there was no significant difference. However, in the protocol-specified sensitivity analysis adjusted for prognostic factors, a significant difference was seen (HR 0.71; 95% CI: 0.55-0.92, p=0.010). In addition, a prespecified per-protocol analysis, excluding the patients ineligible at enrollment and those not receiving capecitabine, showed a significant difference (HR 0.75, 95% CI: 0.58-0.97, p=0.028). The recently reported one is the ASCOT trial comparing S-1 with surgery alone. The primary endpoint was overall survival, and the secondary endpoints were recurrence-free survival and adverse events. The 3-year survival rate was 77.1% in the S-1 group and 67.6% in the surgery alone group, which were significantly better in the S-1 group (HR 0.694, 95% CI: 0.514-0.935, p=0.008). The 3-year recurrence-free survival was 62.4% in the S-1 group and 50.9% in the surgery alone group, which was good in the S-1 group (HR 0.797, 95% CI: 0.613-1.035). **Conclusion:** Based on the results of these two studies, postoperative adjuvant chemotherapy with orally administered fluoropyrimidine agents has been established to be a standard treatment. **Keywords:** resectable biliary tract cancer; adjuvant therapy; fluoropyrimidine agents; capecitabine; standard treatment.

S2-2

Immunotherapy for HCC: current status and future directions

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Immune checkpoint inhibitor (ICI) has changed the landscape of treatment for hepatocellular carcinoma (HCC) in several aspects. First, the use of ICI as monotherapy typically leads to 10–15% radiologic responses in advanced HCC which are usually more durable than conventional TKI. Recent phase III clinical trials also show that the combination of TKI or high-dose bevacizumab with ICI could lead to improved survivals of patients with advanced HCC. Second, the treatment strategy in the intermediate-stage HCC is changing as a result of improvement of systemic therapy. Phase III clinical trials have been initiated to test the addition of ICI to TACE in intermediate-stage HCC. The concept of challenging the TACE with atezolizumab-bevacizumab is also evaluated by an ongoing randomized study. Third, it is potentially feasible that the adjuvant uses of ICI-combination could delay or even prevent the recurrence of HCC after surgery or curative ablation. It is anticipated that trial readouts will be available soon from several completed phase III clinical trials. Future directions of research should be focused on: 1) identification of predictive signature for ICI uses in patients; 2) development immunotherapy apart from ICI (e.g. cellular therapy) and 3) refining the ICI-TKI sequence of treatment for HCC. **Keywords:** hepatocellular carcinoma; immunotherapy; immune checkpoint inhibitor; systemic therapy.

S2-3

What's the role of locoregional therapy in the era of new systemic agents?

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Locoregional therapy (LRT) has been a mainstay in real-world practice for intermediate and advanced HCCs in Asia before TKIs, the best objective response rate (ORR) was higher than that of sorafenib (<10%). Latest 1st line agents including lenvatinib or atezolizumab plus bevacizumab boost the ORR to 70% with considerable complete response (CR) rates and conversion to curative options. Recently, the efficacy of LRT is also improving due to new techniques and technologies (Table 1). LRT can be combined each other to potentiate local tumor control. Therefore, it is more complicated to schedule the order of treatment for intermediate and advanced HCCs because the continuous spectrum in tumoral and patient characteristics, lacking comparative data for specific conditions and institutional variability in availability and expertise of treatment options. There are two major factors determine the survival of HCC patients: tumor control and preservation of liver function. In general, LRT has advantage in local tumor control except far-advanced conditions while systemic therapy in preserving liver function and controlling intrahepatic and systemic

Table 1. LRT and relevant new techniques and technologies

LRT	New techniques and technologies
local ablation	no-touch technique and navigation technology
ultraselective chemoembolization	microcatheter and C-arm cone-beam CT technology
radiation tumorectomy/ segmentectomy/ lobectomy	advanced personalized dosimetry in radioembolization
HAIC	new protocol
radiotherapy	stereotactic radiotherapy; proton-beam radiotherapy

metastasis. In adverse events, systemic therapy may be advantageous in far-advanced or high tumor burden conditions. LRT as the 1st line option will continue to shrink until a well-designed randomized trial demonstrates survival advantage of a LRT or its combination over 1st line systemic options. If the advanced tumor is successfully downstaged through systemic therapy, LRT can be applied to second-line to achieve CR. In applying LRT for intermediate or advanced HCCs with low tumor burden, we should think about effective systemic therapy with high ORR and low risk for liver function deterioration. Therefore, procedures should be performed very selectively to minimize normal parenchymal damage and maximize local tumor control. Early conversion to systemic therapy should be strongly considered before hepatic decompensation when repetitive nonselective procedure is required or the results are not satisfactory. Generally, a combination of LRT and systemic therapy can achieve better tumor response, but toxicity, hepatic damage and cost may also increase. Currently, multiple randomized trials are initiated and ongoing. We will see the results about survival advantage and cost effectiveness soon. **Keywords:** hepatocellular carcinoma; locoregional therapy; systemic therapy; combination; therapy strategy.

S2-4

Precision immunotherapy for HCC and iCCA: where are we in 2022?

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Systemic therapy for HCC has transformed in the last several years beyond oral multi-kinase inhibitors. The first line therapy for advanced HCC with Child's Pugh A now includes Atezolizumab + Bevacizumab, Durvalumab + Tremilumumab, and Sintilimab + Bevacizumab biosimilar, but none of these therapies have a biomarker for improved efficacy. I will discuss more precise immunotherapy against HCC, including: does aetiology matter, PD1 and PDL-1, and recently published predictive biomarkers of IO efficacy in HCC. In advanced biliary tract cancer (BTC), therapeutics beyond cytotoxic chemotherapy has now opened up to both immune and molecular targeting, especially for intrahepatic cholangiocarcinoma (iCCA), which has the highest genetic alterations of all BTC, including IDH1/2, FGFR2, HER2, NTRK. Recent reports highlight compelling clinical efficacy of HER2/neu targeting also. MSS/MSI remains the key biomarker for immune checkpoint inhibitor in iCCA and overall in BTC. The recently published Phase III TOPAZ-1 trial: PDL-1 inhibitor durvalumab added to gemcitabine + cisplatin (GC) in the first line treatment of BTC confirmed significantly improved OS, PFS and ORR. PDL-1 Tumor Area Positivity (TAP) >1% and <1% both reflected benefit for the GC + durvalumab arm so PDL-1 is not a definite biomarker in this study. Numerous IO trials are ongoing in BTC. **Keywords:** hepatocellular carcinoma; intrahepatic cholangiocarcinoma; precision therapy; immunotherapy; targeting therapy.

State-of-the-Art Lecture 2

SL2-1

Chinese guideline for the management of hepatocellular carcinoma: updates and insights in China

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Early this year, we published the Guidelines for the Diagnosis and Treatment of Hepatocellular Carcinoma (HCC) (2022 Edition), the Chinese Liver Cancer (CNLC) staging system. Chinese HCC patients' characteristics differ from those from other countries or regions. Most of them were HBV-related HCC and were diagnosed at an intermediate-advanced stage, and the payment modes are also different. So, we need a CNLC guideline. The major differences between CNLC guideline and other guidelines are: (1) we provide more treatment options for each stage; (2) subdivide the intermediate stage into CNLC IIa (tumor number: 2-3) and IIb stages (tumor number ≥4) and recommend surgical resection for the patient with CNLC IIa stage; (3) subdivide the advanced stage into CNLC IIIa (vascular invasion) and IIIb stages (extrahepatic metastasis) and recommend transcatheter arterial chemoembolization for CNLC IIIa stage. Chinese patients may benefit more from novel systemic therapies, such as the atezolizumab-bevacizumab combination. We have several systemic therapies only in China, including the sintilimab-bevacizumab combination and donafenib as first-line treatment and apatinib, camrelizumab, and tislelizumab as second-line therapy. These agents developed by Chinese pharmaceutical companies were much more affordable. Because most HCCs were unresectable at diagnosis, we performed a more aggressive strategy for selected patients. With some effective conversion therapies, such as Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy (ALPPS) and systemic therapies with or without locoregional therapies, some unresectable HCC were converted into resectable HCC and may have long-term survival. **Keywords:** hepatocellular carcinoma; Chinese guideline; management; updates and insights; treatment option.

Session 3: Advancement of anti-viral treatment in HCC

S3-1

Precision management of chronic hepatitis B

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The association between high HBV DNA levels (>6 $\log_{10}$ IU/mL) and hepatocellular carcinoma (HCC) risk remains unclear, and antiviral treatment of the patients with high HBV DNA and normal ALT levels is controversial. Recently we analyzed the association between HBV DNA levels and HCC risk in 6949 HBeAg-positive and HBeAg-negative, non-cirrhotic, treatment-naïve chronic hepatitis B (CHB) patients who are not generally indicated for antiviral therapy by current guidelines because of normal ALT level.[1] We found the association is parabolic: the risk was highest at moderate HBV DNA levels (6.3 $\log_{10}$ IU/mL) and lowest at very high or very low levels (>8 $\log_{10}$ IU/mL or ≤4 $\log_{10}$ IU/mL). Similar findings were consistently observed in all age subgroups. Our another study demonstrated untreated non-cirrhotic HBeAg-positive CHB patients with persistently normal ALT levels had higher risks of HCC than the immune-active phase patients treated with nucleos(t)ide analogs for elevated ALT levels.[2] Further, untreated non-cirrhotic HBeAg-negative CHB patients with high viral load and no significant ALT elevation had higher risks of clinical events than treated HBeAg-negative active hepatitis phase patients with elevated ALT.[3] Based on the findings, we suggest to use the terms "high replication phase," "moderate replication phase," and "low replication phase" to distinctively indicate HCC risk and the necessity of treatment. Because very high levels may eventually decline and subsequently increase the risk, we suggest patients at very high(especially >30 years old) or persistently moderate viral load (>8 $\log_{10}$ IU/mL or 4–8 $\log_{10}$ IU/mL) may be indicative of antiviral treatment. We found on-treatment HCC risk increased with decreasing baseline HBV DNA levels in the range of ≥5.00 $\log_{10}$ IU/mL in HBeAg positive, non-cirrhotic CHB patients.[4] Therefore, early initiation of antiviral treatment with a high viral load (≥8.00 $\log_{10}$ IU/mL) may maintain the lowest risk. We also found starting antiviral therapy in immune-tolerant phase is cost-effective compared with active hepatitis phase, especially with increasing HCC risk, decreasing drug costs and consideration of productivity loss.[5] Simplifying and expanding treatment eligibility would save many lives and be highly cost-effective when combined with high diagnostic rates.[6] Collectively, it may should be recommended to initiate treatment in CHB patients >30 years with HBV DNA >2,000 IU/mL. **Keywords:** chronic hepatitis B; HBV DNA level; hepatocellular carcinoma; association; antiviral therapy.

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S3-2

Novel biomarkers for HBV infection

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Chronic hepatitis B (CHB) infection leads to clinically heterogeneous disease outcomes across the world, and several existing viral markers have been used to diagnose, monitor treatment responses and predict risk of disease progression to liver cirrhosis/hepatocellular carcinoma (HCC) in CHB patients. On the basis of innovations in molecular medicine and genomics, novel hepatitis B viral and host biomarkers associated with diagnosis and disease progression of chronic hepatitis B virus (HBV) infection have been elucidated. For large-scale screening of chronic hepatitis B (CHB), point-of-care tests provide simple and rapid methods for HBV detection. Regarding the monitoring of CHB progression, hepatitis B core-related antigen (HBcrAg) and HBV RNA are positively correlated with intrahepatic cccDNA. The serum HBcrAg level is a predictor of cirrhosis and HCC development, whereas HBV RNA is associated with the response of antiviral therapy and can serve as a biomarker to predict viral relapse after discontinuation of antiviral therapy. Total anti-HBc level may reflect the host immune response to HBV infection and is significantly correlated with the severity of hepatic inflammation and fibrosis as well as HBsAg seroclearance. For HCC surveillance, Mac-2 binding protein glycosylation isomer (M2BPGi) is associated with HCC development in treatment-naïve patients and at virological remission in those on antiviral therapy. In conclusion, these novel biomarkers help clinicians monitor the natural course and treatment response of CHB. The combination of novel and already in use biomarkers will improve the ability of early diagnosis and prognostic prediction of HBV-related HCC. **Keywords:** chronic hepatitis B; biomarkers; serum HBcrAg; HBV RNA; anti-HBc level.

S3-3

The safety of stopping nucleos(t)ide analogue in chronic hepatitis B

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Background: Recent studies suggest stopping nucleos(t)ide analogues (NUCs) after at least 2 years treatment and undetectable serum HBV DNA may result in higher rates of HBsAg seroclearance. The 8-year cumulative rates of HBsAg loss were nearly 20%. **Methods and Results:** Three prospective randomized controlled trials (RCTs) were conducted to evaluate the benefits and harms of stopping NUCs. The FINITE study assigned 42 non-cirrhotic HBeAg-negative German patients who had received tenofovir disoproxil fumarate (TDF) for at least 4 years to stop or continue. HBsAg seroclearance at 2 years was 19% in patients who stopped compared to 0% in those who continued. Only 2 patients restarted treatment and none experienced hepatic decompensation. The Toronto STOP study enrolled 67 patients, and most were receiving TDF and mean duration of treatment was 7.3 years. They were randomized 2:1 to stop or continue NUCs. 27 patients were HBeAg positive and undergone seroconversion for 4 years before enrollment. At 72 weeks, only 2 patients had HBsAg seroclearance, and 38% of those who stopped resumed treatment. In the Stop-NUC trial, 158 German patients who had received NUCs for ≥4 years were randomized 1:1 to continue or stop. At 96 weeks, 10% of who had stopped and 0 of continued achieved HBsAg seroclearance, only 14% resumed treatment. The reasons causing differences in outcomes of these similarly-designed trials might be race and the duration of infection. 93% patients in Toronto trial were Asians while the other trials enrolled mostly (>85%) Caucasians. A international multi-center retrospective study of 1,541 patients found the 4-year cumulative rate of HBsAg seroclearance was 14% and Caucasian was the only independent predictor of HBsAg seroclearance (HR 5.8, 95% CI: 3.6-9.5). Moreover, 40% of the Toronto trial patients were HBeAg positive before treatment while all the rest were negative. **Conclusion:** Collectively, these studies suggest stopping NUCs after >4 years treatment in HBeAg-negative patients might be associated with higher rates of HBsAg seroclearance compared to continuing, but the rates varied from 2.7-16.7%/year in Caucasian and are lower in Asian patients, it may only be appropriate for specific patients with no cirrhosis before treatment but not for Asians. The widely accepted predictor of functional cure is low HBsAg level at the time of NUC withdrawal. **Keywords:** chronic hepatitis B; stop nucleos(t)ide analogue; safety; HBsAg seroclearance; Caucasian.

S3-4

Diagnosis and treatment of hepatitis B and C

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Chronic hepatitis B virus (HBV) infection is a worldwide public health problem. The HBsAg prevalence in China mainland has reduced to 6.1% in the general population, nevertheless, there still are around 80 million persons living with chronic HBV infection, which carries a heavy HBV-related disease burden. Antiviral therapy with the first-line nucleos(t)ide analogs (NAs) is very effective in reducing the burden including cirrhosis, hepatocellular carcinoma(HCC) etc. Therefore, promoting screening, diagnosis and treatment of HBV is the key to inverting the premature deaths caused by CHB. However, due to insufficient awareness and issues of treatment cost and availability, the rate of diagnosis and treatment are still low, and the prescription of the recommended agents is insufficient. To improve the accessibility and affordability of medicines, China issued the National Centralized Drug Procurement (NCDP) policy in 2019, as a result, the price of the first-line anti-HBV agents significantly decreased, and the usage dramatically increased. As an example, significant increases in diagnosis and antiviral therapy of CHB were observed from 2010 (when anti-HBV agents started to be included in the reimbursement list) to 2018 in Beijing. Furthermore, basic medical insurance coverage of first-line anti-HBV medications in Beijing significantly reduced the risk of liver-related death from 0.38% to 0.16% for non-cirrhotic CHB patients and from 4.03% to 3.39% for those with compensated cirrhosis. These favorable changes will translate into long-term clinical and public health benefits. However, there is still a big gap to the target of 80% treatment rate to achieve the goal of the World Health Organization. Chronic hepatitis C virus (HCV) infection is also a critical public health issue. The prevalence of HCV infection has declined to around 1% now in China due to the implementation of strict control of blood donation and transfusion, manufacture and use of blood products, and the safe injection. The highly efficacious direct antiviral agents (DAAs) have been proved and included in the reimbursement list after massive price reduction by governmental negotiation. However, the challenging issue is the low rates of diagnosis and treatment. Raising awareness of HCV through public education, advocacy for the massive test and find-and-treat strategy would contribute to achieving the goal of eliminating viral hepatitis by 2030. **Keywords:** chronic hepatitis B; diagnosis; antiviral therapy; China; chronic hepatitis C.

Session 4: Surgical treatments for liver cancer

S4-1

Future perspectives on the surgical treatment of HCC

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In the West, hepatitis C virus (HCV) has been the most common liver disease underlying hepatocellular carcinoma (HCC). Because patients with HCV-related HCC commonly have cirrhosis with compromised liver function, liver transplantation (LT) is often required to remove early HCC. In an analysis of our center, 75% patients undergoing surgery for early-stage HCV-HCC underwent LT through 2015, compared to 33% with HBV-HCC. After steadily rising through the early 21st century, HCC incidence

in the US, and HCC-related mortality, began to fall in 2014 coincident with the introduction of direct-acting antiviral (DAA) therapy. As a result, UNOS Transplant Registry data indicate a steady drop in the number of LT for HCC; 57% of LT for HCC at Mount Sinai in 2014 whereas 28% in 2020. The number of HCC resections has also fallen from 100 in 2011 to 40 in 2019. This reflects a decreasing incidence of viral hepatitis-related HCC because of the broad use of antiviral therapy in patients with chronic hepatitis before cirrhosis and increased adoption and effectiveness of curative nonsurgical treatments for HCC including microwave ablation, radioembolization, and stereotactic body radiotherapy. As viral-related HCC declines, HCC related to fatty liver disease, both alcoholic (ALD) and nonalcoholic (NASH), is rapidly becoming an important underlying etiology for HCC. In our overall experience 49% of HCC has been due to HCV, 7% to ALD, and 8% to NASH; among patients first seen in 2019, 41% had HCV, 12% ALD, and 17% NASH. Patients with ALD/NASH are, as a group, suboptimal candidates for resection. On the other hand, ALD/NASH is the fastest-rising etiology of HCC among LT patients in the US: at Mount Sinai whereas overall 15% of LT patients has ALD/NASH, in 2019-2020 the proportion rose to 33%. Patients with NASH-HCC tend to be older than those with viral-elated HCC and, since NASH is a manifestation of systemic disease (metabolic syndrome), they have more comorbidities. It is not surprising that data suggest LT outcomes are not as good with NASH-HCC as compared to other etiologies. As a result of these multiple developments in demographics and the treatment of HCC, the US transplant community has reduced the priority given to HCC patients on the LT waiting list. Immunotherapy is now the cornerstone of systemic therapy. Having been shown to significantly improve survival in advanced HCC, it is being introduced earlier in the neoadjuvant/adjuvant setting. Regardless of surgical advances, HCC recurrence following resection is common since vascular invasion and tumor dissemination begins at early-stage. Survival improvement depends on ways to eliminate micrometastatic disease, and trials are underway using immune checkpoint inhibitors before and after resection. **Keywords:** HCV-HCC; HBV-HCC; surgical treatment; liver transplantation; HCC resection.

S4-2

ICG-guided laparoscopic anatomical liver resection

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Background: Anatomical resections (ARs) have theoretically better short-term outcomes than partial resections in patients with hepatocellular carcinoma (HCC) and colorectal liver metastases (CRLM). Few experiences with laparoscopic anatomical resections have been reported, and no data on little AR exist. This presentation aimed to describe ICG-guided laparoscopic anatomical parenchymal sparing liver resections for HCC and CRLM. **Methods:** We routinely perform laparoscopic anatomical parenchymal sparing liver resections using ICG with preoperative surgical simulation and standardized procedures. **Results:** A total of 121 patients included 52 HCC, 51 CRLM, 10 CCC, and eight others. Twelve anatomical subsegmentectomies, 54 segmentectomies, 30 mono-sectionectomies, 24 bi-sectionectomies, and one trisectionectomy were performed. The difference between preoperative 3D simulation (predicted resection volume) and actual resection weight was less than 10% in 84.0% of these anatomic resections. Thirteen patients experienced complications over CD IIIa. The mean operative time was 354 min, and the mean blood loss was 300 ml. **Conclusions:** Precise preoperative planning and an ICG-guided standardized surgical technique allow us to pursue the specific AR, enhancing the safety of the parenchyma-sparing principle with good short-term outcomes. **Keywords:** liver resection; hepatocellular carcinoma; colorectal liver metastases; laparoscopic anatomical resection; ICG.

S4-3

ALPPS treat traditionally-unresectable HCC

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Background: Insufficient future liver remnant is an important factor that precludes patient from upfront major liver resection as it predisposes to developing post-hepatectomy liver failure and mortality. As such, augmentation of future liver remnant (FLR) by portal vein embolization (PVE) was the conventional approach to improve the safety profile of major hepatectomy. In recent years, associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) has been popularized as an alternative approach for FLR augmentation. Through the establishment of the international ALPPS registry and familiarization of the procedure, there has been an improvement in the short-term outcome resulting in an inter-stage morbidity and 90-day mortality rate from 10% to 3%, and from 17% to 4% respectively. Nonetheless, most of the evidence in the literature focus on the applicability of ALPPS procedure for bilobar colorectal liver metastases, or other non-hepatitis related liver tumors. Results on the short-term as well as oncological outcome of ALPPS procedure solely for hepatocellular carcinoma (HCC) due to viral hepatitis remained relatively sparse. **Methods:** We started to perform ALPPS for hepatitis-related HCC in 2013 and until, almost 100 cases have been accumulated. 54.4% of patients had chronic hepatitis, predominantly hepatitis-B infection (89.1%), the remaining patients had histologically proven cirrhosis. The indocyanine green clearance rate was 10.1% and the median tumor size was 8.5cm, predominantly solitary tumor. After stage I ALPPS, the absolute FLR volume increment was 48.8% over a median of 6 days. Open procedure remained the standard approach for both stages of ALPPS. **Results:** The overall morbidity and mortality rates were 20.5% and 6.5% respectively. The 1-, 3- and 5-year disease-free survival rates were 63.2%, 34.9%, and 25.0%, and the 1-, 3- and 5-year overall survival rates were 84.7%, 60.2%, and 46.8%. **Conclusion:** Our experience showed that ALPPS remained effective to induce rapid FLR hypertrophy in hepatitis-related HCC with satisfactory long-term survival. **Keywords:** unresectable hepatocellular carcinoma; ALPPS; future liver remnant; hepatitis-related HCC; long-term survival.

S4-4

Fluorescence-guided surgery cancer surgery for hepatobiliary diseases

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Recently, intraoperative fluorescence imaging has been used in a variety of surgical fields for the following purposes: 1) tissue perfusion assessment, 2) cancer identification, 3) lymphatic system mapping, and 4) anatomy visualization. In the field of hepatobiliary surgery, fluorescence imaging using indocyanine green (ICG) can be applied for real-time visualization of the bile ducts, liver cancers, and hepatic segments. For the visualization of the bile ducts (fluorescence cholangiography), ICG (2.5 mg) is injected intravenously before or during surgery and its biliary excretion can be used as a fluorescence source. In order to identify liver cancers, liver function test by intravenous administration of ICG (0.5 mg/kg, ICG retention rate at 15 min) is usually performed 2-7 days prior to surgery. Then, subcapsular tumors can be visualized by intraoperative fluorescence imaging because preoperatively-injected ICG accumulates in cancerous tissues of differentiated-type hepatocellular carcinoma (HCC) and in non-cancerous hepatic parenchyma surrounding poorly-differentiated HCC, intrahepatic cholangiocarcinoma and colorectal liver metastasis. For hepatic segmentation, ICG solution (0.25 mg/5 mL) is injected into the portal branch of tumor-bearing hepatic segment under ultrasound guidance (positive staining technique). Hepatic segments can also be identified as non-fluorescing regions of the liver by intraoperative intravenous injection of ICG (1.25-2.5 mg) following closure of the corresponding portal pedicles (negative staining technique). In these years, consensus guidelines on a use of intraoperative fluorescence imaging have been created by international study groups. Some studies have also suggested real advantages of fluorescence-guided hepatobiliary surgery from oncological aspects as well as in terms of intraoperative and short-term outcomes. **Keywords:** unresectable hepatocellular carcinoma; ALPPS; future liver remnant; hepatitis-related HCC; long-term survival.

Session 5: Molecular mechanisms and pathogenesis of HCC and iCCA

S5-1

Targeting myeloid-derived suppressor cells in the treatment of hepatocellular carcinoma

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Myeloid-derived suppressor cells (MDSCs), a heterogeneous population of immature myeloid cells with various states of differentiation, play an important role in the development and progression of cancers by promoting immunosuppression and tumor angiogenesis. Previous clinical studies of hepatocellular carcinoma (HCC) patients, most of them involving detection of MDSCs in peripheral blood, have found that the frequency of MDSCs was increased in HCC patients, high frequency of MDSCs was associated with adverse clinical features of HCC, and low frequency of MDSCs correlated with improved outcomes in HCC patients receiving various types of locoregional therapies. Preclinical studies using mouse liver cancer models have demonstrated that MDSCs not only contributed to tumor progression, but also attenuated the therapeutic efficacy of multikinase inhibitors such as sorafenib or immune checkpoint inhibitors in experimental animals. Targeting MDSCs can be achieved by (1) reducing the generation and survival of MDSCs, (2) blocking the trafficking and migration of MDSCs to tumor microenvironment, (3) inhibiting the immunosuppressive function of MDSCs, and (4) inducing the differentiation and maturation of MDSCs. These MDSC-targeting strategies, although demonstrated potential therapeutic efficacy in preclinical studies, have not yet proven to be clinically useful in the treatment of patients with HCC. Future clinical and translational studies are warranted to prove the usefulness of targeting MDSCs, alone or in combination, in the treatment of human HCC. **Keywords:** hepatocellular carcinoma; myeloid-derived suppressor cells; treatment; targeting MDSCs; mechanism.

S5-2

HCC-genomics for translating into biomarker discovery

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Our increasing understanding of Hepatocellular carcinoma (HCC) biology holds promise for personalized care and in the development of molecular drugs. However, HCC is characterized by significant inter-tumoral heterogeneity between patients as well as intratumoral heterogeneity within the same tumor of an individual patient. The molecular alterations in advanced HCCs are quite diverse and HCC is characterized by considerable molecular and genetic heterogeneity. This presentation attempts to highlight the recent advances in finding and identifying the molecular targets or signature of HCC for biomarkers in HCC for better patient stratification for treatment and patient management. Recent data will be discussed to demonstrate how genomic/genetic analysis of patients' HCC samples can help identify important molecular targets and molecular biomarkers or signatures of HCC for effective treatment. There is also emerging evidence supporting the importance of tumor microenvironment in providing a favorable and supportive niche to expedite HCC development. Studies of the immune tumor microenvironment proposed different classes of HCC patients according to the expression signatures of immune cells. Very recently, the cutting-edge technology of single cell analysis has provided important information of its intratumoral heterogeneity and is able to identify important sub-populations, including immune landscape. Discoveries and insight into the complex pathways have created opportunities for molecular targets and biomarkers and new therapeutic approaches for this malignant disease. **Keywords:** hepatocellular carcinoma; biology; genomic/genetic analysis; molecular target; molecular biomarker.

S5-3

Mechanisms of defective hepatic autophagy in liver injury and tumorigenesis

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Background: Non-alcoholic and alcoholic liver disease and viral hepatitis are common chronic liver diseases and risk factors of hepatocellular carcinoma, which are often associated with impaired hepatic autophagy with increased mechanistic target of rapamycin (mTOR) activation. Autophagy is a lysosomal degradation pathway that degrades cytoplasmic proteins and organelles, which is critical to maintain cellular homeostasis and functions. **Methods:** Over a decade research in my lab using multiple genetic engineered mice that are defective of hepatic autophagy, nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and mTOR, we dissected the complex interplay among mTOR, autophagy, p62/SQSTM1 and Nrf2 on liver cell repopulation, metabolic reprogramming, and redox homeostasis in liver tumorigenesis. **Results:** Our results uncovered an unexpected dual role of mTOR and p62/SQSTM1 in liver tumorigenesis. **Conclusion:** Results from our findings may provide the basis on targeting Nrf2 and mTOR for liver cancer therapy by improving the liver cell repopulation and metabolic reprograming in particular in a subset of HCC with mTORC1 hyperactivation and defective autophagy. **Keywords:** hepatocellular carcinoma; autophagy; mechanistic target of rapamycin; nuclear factor (erythroid-derived 2)-like 2; p62/SQSTM1.

S5-4

Epigenetics links intermediary metabolism and gene regulation

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How cells coordinate metabolic, epigenetic, and signaling pathways for survival and growth is a fundamental question of biological research. Increasing evidence suggests that the cellular transcriptional machinery and its chromatin-associated proteins integrate metabolic signals to mediate homeostatic or adaptive responses. Chromatin senses metabolic status and converts epigenetic information to specific transcriptional outputs, which is crucial for homeostatic or adaptive responses in the cell. The discovery of three oncometabolites (2-HG, succinate, and fumarate) has led to the current view that endogenous metabolites can convey oncogenic signals to regulate chromatin-modifying enzymes, such as α-KG/Fe (II)-dependent KDM and TET enzymes, and drive epigenetic gene regulation. This is a fascinating example for how epigenetics links intermediary metabolism and gene regulation. Nevertheless, characterization of specific mechanisms that coordinate metabolic, epigenetic, and signaling pathways is challenging and many fundamental questions persist. Besides known oncometabolites, there are more endogenous metabolites which when highly accumulated, can act as α-KG antagonist(s) in a specific cellular context. The growing field of immune metabolism has revealed promising indications for metabolic targets to modulate anti-cancer immunity. Will intervening metabolism-epigenetic axis in immune cells have synergistic effects and improve ongoing immunotherapeutic strategies, especially in solid tumors? Answers to this question will lead to new mechanistic and clinical understanding of the crosstalk between metabolism and epigenetic regulators and, possibly, bring new therapeutic opportunities. **Keywords:** metabolic; epigenetic; transcription; signal; intermediary metabolism.

Session 6: Liver transplantion for HCC

S6-1

Innovations in pediatric transplantation

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As estimates of 30 year survival after pediatric liver transplantation for patients transplanted in the more recent era (>2007) have now reached 80%, increasing focus has turned to identifying the current barriers to further improving outcomes which include elimination of wait list and pre-transplant mortality, reducing intraoperative and perioperative complications, enhancing early graft function and optimizing sustainable health. Important technological advances such as minimally invasive surgical approaches or applications of machine learning, health learning systems, immunologic diagnostics are impacting each of these areas. Key to the elimination of waitlist outcomes is proper graft selection and the utilization of both technical variant and living donor (LD) grafts. In the United States, deceased donor (DD) grafts continue to be more commonly employed than LD grafts. LD grafts have demonstrated excellent early and long-term results both in single center and meta-analysis. Machine learning approaches are beginning to be applied in decision support tools in organ selection, to prediction of post-transplant outcomes as well as to prediction of tumor recurrence post transplantation, a topic of particular importance in this Congress. Technical innovations to optimize donor recovery and minimize perioperative complications have included significant progress in minimally invasive donor hepatectomy as well as innovative techniques to address problems unique to pediatric transplantation such as atretic portal venous anatomy. Optimizing long term graft function continues to focus on understanding the challenges of late graft fibrosis. Studies of tolerant patients as well as prospective immunosuppression withdrawal trials have yielded insight into some potential mechanisms, but no actionable prospective mechanisms as of yet. Immune monitoring include cell free DNA is being studied in pediatric populations along with implications of donor specific antibodies, both of which have been studied in greater degree in the renal population. Finally, learning health systems such as the Starzl Network for Excellence in Pediatric Transplantation have arisen to address a key fundamental need in the field for more rapid dissemination of best

practices and new knowledge. The Starzl Network targets key "pain points" in transplantation such as immunosuppression, perioperative events, quality of life and transition all of which have been identified as key to optimizing long term outcomes. **Keywords:** transplantation; pediatric; innovation; technological advance.

S6-2

Bridge or downstage locally advanced hepatocellular carcinoma to living donor liver transplantation

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With the recent advances in surgical techniques, locoregional and medical therapies in the management of HCC, prognosis of patients with HCC has tremendously improved. Liver transplantation has undoubtedly become the definitive standard treatment in providing the longest overall and recurrence-free survival when performed within one of many validated criteria. In Asia, where LDLT has been more widely accepted and has flourished, we have continuously explored and produced innovative surgical techniques that have effectively expanded not only the donor pool but likewise extended recipient indications for LDLT. In recent years, rather than excluding HCC with unfavorable histopathology, we have started to selectively utilize proton beam or Yttrium-90 radioembolization as an alternative locoregional therapy to bridge or downstage locally advanced or aggressive HCC to improve recurrence-free survival. Our experience has demonstrated that downstaged HCC patients have similar survival outcomes to that of patients who initially fit the criteria. Attempts to achieve complete pathological response by loco-regional therapy before transplant may further improve recurrence-free survival. Powerful modern locoregional therapies like proton beam and Y-90 together with target and/or immunotherapy may effectively bridge or downstage locally advanced or aggressive HCC in preparation for timely LDLT, with promising survival outcomes in patients with otherwise dismal prognoses. **Keywords:** locally advanced hepatocellular carcinoma; bridge; downstage; locoregional therapy; living donor liver transplantation.

S6-3

Minimally invasive donor hepatectomy for living donor liver transplantation

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To overcome a shortage of deceased organ donation, living donor liver transplantation (LDLT) has been developed and accepted as an alternative to deceased donor liver transplantation (DDLT) for patients with end-stage liver disease. Unlike DDLT, the safety and requirements of the live donor should also be considered in LDLT. As experience grows and surgical techniques evolve, pure laparoscopic hepatectomy has become a new option considering the donor's increasing cosmetic and functional demands. However, following the first report of pure laparoscopic living donor left lateral sectionectomy in 2002, still limited number of studies reported the results of pure laparoscopic left lateral sectionectomy and left hepatectomy. Furthermore, very few centers with significant experience have reported the results of pure laparoscopic living donor right hepatectomy limited to case report or case series. Since the initiation of our LDLT program in January 1999, more than 1200 LDLTs have been performed in our center and most of the grafts were right lobe to balance the demand of the recipient and safety of the donor. There were no donor deaths, no disabling morbidities, and no transfusions until now. After first two cases of hand-assisted laparoscopic living donor right hepatectomy in 2007, laparoscopy-assisted technique was used in a small number of donors who met strict criteria. Since introducing flexible 3-dimensional laparoscope into liver surgery in 2015, laparoscopy-assisted technique was more frequently used in donor hepatectomy and in November 2015, first pure laparoscopic donor right hepatectomy was performed. We performed pure laparoscopic donor hepatectomy in selected donors with no anomalies of the bile duct or portal vein until February 2016. However, since March 2016, with accumulation of experience and introduction of indocyanine green (ICG) near-infrared fluorescence camera for real-time demarcation and cholangiography, more than 90% donor hepatectomies were performed using pure laparoscopic method without any special selection criteria. **Keywords:** living donor liver transplantation; pure laparoscopic; minimally invasive.

S6-4

Novel surgical and immunological aspects for treatment of HCC

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Surgical resection or interventional destruction of the tumor or liver transplantation are the only therapeutic option for cure of the patient. Moreover, optimized diagnostics, additional treatment modalities aiming at pro- or anti-angiogenic modalities as well as at immune responses against the tumor may contribute to cure.

Innovative approaches for treatment, therefore, aim at:

A. using intraoperative contrast-enhanced ultrasound (CEUS) for highly sensitive detection of tumor lesions in the liver;
B. increasing resectability of larger tumors by innovative techniques like ALPPS and other two-stage techniques;
C. performing liver transplantation with reduced donor risk by a two-stage transplantation technique using the left lateral donor liver lobe only,
D. using irreversible electroporation as a non-thermic ablation method for tumors adjacent to relevant vascular or biliary structures – alternatively or in addition to surgical resection,
E. selecting the immunosuppressive treatment after liver transplantation to prevent pro-angiogenic and promote anti-angiogenic factors, and

F. analyzing the intra- and peritumoral immune infiltrates of HCCs during resection and transplantation as basis for development of novel immune therapeutic approaches.

Examples for the above approaches will be presented in the lecture. The therapy of patients with HCC requires a broad toolbox of diagnostic and therapeutic methods and each patient requires a highly individualized treatment concept depending on many variables, which are – in our opinion – not adequately reflected by the broadly used BCLC-classification. Treatment of HCC patients should always be performed is highly specialized centers for optimal treatment results. **Keywords:** HCC; surgical; innovative approach; immune therapeutic approach; additional treatment modalities.

Session 7: Updates of imaging diagnosis: from image to prognosis

S7-1
Imaging diagnosis of early stage HCC

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Overcoming hepatocellular carcinoma (HCC) is still challenging. Hence, we properly need to understand imaging diagnosis of HCC. The development from chronic hepatitis to HCC involves a process of multistep carcinogenesis, total amount of arterial blood flow decreases slightly between the stages of high-grade DN and early HCC, and increases in progressed HCC. Thus, HCC can show either hypo or hypervascularity during hepatocarcinogenesis. In AASLD guideline, early stage HCC is solitary HCC or 2-3 small HCCs less than 3cm that can be consisted of hyper- or hypovascular HCCs. Multiphasic dynamic contrast-enhanced CT and MRI studies are essential in the detection and characterization of HCC. Hyperenhancement in the arterial phase, washout of contrast medium in the portal venous and equilibrium phases of the dynamic studies, and hypoenhancement in the hepatobiliary phase of Gd-EOB-DTPA are especially very important findings of early stage HCCs. Gd-EOB-DTPA helps in the more accurate detection of hypervascular HCC than other extracellular contrast agents and can detect hypovascular HCC, an early stage of carcinogenesis. Gd-EOB-DTPA uptake starts to decrease earlier than the other events in hepatocarcinogenesis, and uptake of Gd-EOB-DTPA is more sensitive to detect hypovascular well differentiated HCC than other imaging features. Hence, Gd-EOB-DTPA MRI is the best imaging modality for detecting early HCC. Hypovascular nodules are often clearly visualized on hepatobiliary phase of Gd-EOB-DTPA MRI, even though they are around 10mm in diameter. They may include regenerative nodule, low/high grade DN and early HCC. Some hypovascular and hypointense nodules on EOB-MRI may show hypervascularization during follow up. High intensity of T2WI shows higher incidence of hypervascularization. Higher growth rate is also risk factor of hyper vascularization. So, to treat hypovascular HCC within early stage, less than 20mm, hypovascular lesion more than 15 mm and lesion less than 15mm with these risk factors should be considered biopsy. On the other hand, we occasionally encounter specific type of HCCs that show high signal intensity in the hepatobiliary phase images, which is associated with β-catenin mutation. Wnt/β-catenin activated/mutated type is likely to be immune cold. It was reported that both PFS and time to nodular progression after immune checkpoint inhibitor (ICI) treatment were significantly shorter in tumors with high intensity on hepatobiliary phase than low intensity. Gd-EOB-DTPA may be a predictive biomarker for response of ICIs. There are hyper- and hypo-vascular nodules in early stage HCCs, which defined as small number and size of HCC. We must know optimal examination and perform precisive follow up. **Keywords:** hepatocellular carcinoma; early stage; imaging diagnosis; MRI; Gd-EOB-DTPA.

S7-2
PET imaging in diagnosis and treatment of liver cancer

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It is well known that ^{18}F-FDG PET in primary liver cancer faces some challenges, such as: It's difficult to show the tumor with slightly ^{18}F-FDG accumulation as liver is an organ of glycogen storage. Most of the liver or bile duct tumor uptake ^{18}F-FDG limited. Liver moves with respiratory movement lead to PET imaging become unclear without gating. The PET and CT or MR image could not be registered very well. With the progress of PET instrument, a novel approach gave the answer to meet the request of liver cancer diagnosis or evaluation. For example, Advanced PET instruments: PET/MR, Long axial field view PET/CT were used in clinical. At the same time multi radiotracer, such as 18F-FDG, 68Ga-DOTA TATE, 68Ga-FAPI become the clinical routine improve the ability of PET to serve the clinical needs. By some typical cased were introduced, we conclude that progress of PET/CT and PET/MR improve the imaging quality and diagnostic performance of primary liver tumor. Multi-radiotracer applied in clinical practice, PET/CT and PET/MR could tell the most important tumor biological information for diagnosis or treatment decision. **Keywords:** liver cancer; imaging diagnosis; novel approach; PET/MR; PET/CT.

S7-3

Medical image analysis and its application in the diagnosis of liver cancer

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The report is about the application of ultrasound imaging in liver cancer diagnosis, and its clues are the different AI techniques used in ultrasound modalities in liver cancer diagnosis and treatment. It mainly includes static images, contrast grayscale videos, radio-frequency (RF) raw signal, and finally summarizing AI diagnostic models. First, diagnosis is based on static ultrasound images. Artificial intelligence is data dependent, there are many clinical problems that cannot provide big data for modeling, and we propose a sparse representation radiomics system to solve them. The sparse representation theory can provide theoretically best results in three aspects: feature extraction, feature screening, and model building, and successfully solve the overfitting problem under small samples by sparse constraints. The first part of the work is to employ a new ultrasound instrument capable of providing viscous imaging. To evaluate the value of this new modality for diagnosis and clinical questions including liver cancer diagnosis, differential diagnosis, and immunohistochemical marker prediction. The second problem is the prediction of primary hepatocellular carcinoma in patients with hepatitis B. The problem addressed is the establishment of AI models under extremely unbalanced sample conditions. Because of the low percentage of HCC occurrence among HBV patients, equal-probability round-robin sampling and online data augmentation were designed to balance the number of HCC and non-HCC samples, which expands the diversity of data and approximates the balance of input samples to ensure the stability of the AI model. The third part of the work is tracking and segmentation of the liver video. The completion of the liver grayscale and imaging video tracking is to segment and track the tumor and to automatically quantify the rich perfusion information in the arterial, portal, and delayed phases. A multistream similarity learning network is proposed to utilize the feature information of the first frame and that of the previous frame in tracking process to better track the current frame, resulting in a high robustness to changes in appearance. The proposed automatic video tracking model provides a powerful tool for the extraction of liver perfusion features. The ultrasound RF signal contains rich information of acoustic impedance, phase, frequency deviation, wave velocity, etc. Besides, because the RF signal is not affected by the complex and diverse signal post-processing in ultrasound imaging, it is expected to provide a solution to normalize ultrasound data and thus solve the difficulties in the development of ultrasound big data. In the fourth part of the work, we established a quantitative model based on ultrasound RF signals to assess the percentage of liver fibrosis. **Keywords:** liver cancer; diagnosis.

S7-4

Targeted-immunotherapy for advanced-stage intrahepatic cholangiocarcinoma and the MRI evaluation

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Intrahepatic cholangiocarcinoma (ICC) is the second most common primary liver cancer, which is highly lethal hepatobiliary malignancy with poor prognosis. Most ICCs are diagnosed at advanced stage and only up to 20% are eligible for surgery once confirmed, and overall survival time was less than 12 months for unresectable ICC, decreased to 3-6 months when untreated. Systemic therapies so far become the first-line regimen in advanced ICC and the combination of targeted and immunotherapy showed an improved therapeutic effect in our hospital. The main problem still meets frequent primary or secondary resistance and nondurable response, so we performed the retrospectively investigation in order to predict the efficacy of combined targeted-immunotherapy in advanced ICC by estimating therapeutic response and the risk of early progression using conventional DCE MR. **Keywords:** intrahepatic cholangiocarcinoma; combination; immunotherapy; systemic therapy.

State-of-the-Art Lecture 3

SL3-1

Surgical innovations in hepatobiliary diseases

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During the past two decades, there has been several innovations developed in the field of hepatobiliary surgery. I would like to cover a few of them to show how these techniques or modalities contributed to safe and precise surgical procedures. **Three-dimensional (3D) simulation and navigation:** 3D simulation software was first launched around the year 2000, allowing surgeons to perform virtual hepatectomy (VH) prior to surgery. Apart from allowing impressive 3D visualization of the intrahepatic vessels, this technique offers a great advantage over conventional preoperative evaluation in calculating the volumes of the vascular territories. Volumetry based on the portal blood supply allows detailed estimation of the volume of Couinaud's segments or even smaller subsegments that cannot be calculated by the conventional hand-trace method. Estimation of the venous drainage area in the living donor liver transplantation setting was the most important application because 3D simulation quantifies the negative impact of venous congestion following sacrifice of the hepatic veins. This technique offers a chance for radical hepatectomy in HCC patients with impaired liver function and CRLM patients with advanced

tumors, without compromising survival. **Fluorescence imaging:** Clinical use of ICG fluorescence imaging in surgery started with angiography during coronary artery bypass grafting and was followed by sentinel node navigation in breast cancer surgery, and intraoperative angiography for cerebral aneurysm. In the late 2000s, Japanese surgeons started to use this imaging modality to visualize hepatobiliary structures, including the hepatic arteries, liver segments, and the biliary tree. Near-infrared imaging systems became commercially available for open surgery in 2005 and for laparoscopic surgery in 2011. The development of more sophisticated and user-friendly fluorescence imaging systems further accelerated their use in liver surgery, including laparoscopic or robotic cholangiography, identification of liver segments, diagnostic imaging for liver cancers, and evaluation of blood flow in the liver. **Keywords:** hepatobiliary surgery; innovation; three-dimensional simulation and navigation; fluorescence imaging.

Session 8: Multidisciplinary approach in liver cancer management

S8-1
Multidiciplinary Management of HCC

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This talk discussed the role of immunotherapy in the multidisciplinary management of primary hepatocellular carcinoma. Major landmark immunotherapy studies either monotherapy, in combination with target therapy or trans arterial chemotherapy in the advanced, intermediate, and resectable HCC treatment were summarized. **Keywords:** primary hepatocellular carcinoma; HCC; multidisciplinary management; immunotherapy.

S8-2
Multidisciplinary therapies for intrahepatic cholangiocarcinoma

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Intrahepatic cholangiocarcinoma (ICC) accounting 5% of primary liver cancer has more invasive and metastatic biological behavior compared to hepatocellular carcinoma (HCC). ICC sometimes invades Glissonean pedicles, hepatic veins, or hepatic hilum. One third of surgical cases accompanies nodal metastasis. In the Japanese TNM-based staging of ICC, T factor includes 1) tumor number, 2) tumor size, and 3) vascular or biliary invasion. Solitary ICC involving the hepatic hilum with nodal metastasis can be estimated in Stage IVA, and 5-year survival rate of surgical patients with Stage IV ICC was 20% in Japanese national data. According to the treatment algorithm for ICC in the Japanese guideline published in 2020, surgical resection can be optimally indicated in patients with single nodular ICC without nodal metastasis. Surgical resection includes hepatectomy with/without biliary resection and nodal dissection. On the other hand, in patients with multiple ICCs with nodal metastasis or distant metastasis, systemic chemotherapy for biliary cancer is recommended. Systemic chemotherapy includes GC (Gem+Cis), GS (Gem+S-1), and GCS (Gem+Cis+S-1) therapy. Chromosomal translocations of FGFR2 can been found in 5-10% of ICC, thus pemigatinib, an anti-FGFR2 drug, might be an alternative anti-cancer drug. Radiation therapy can be an option for unresectable ICC. A Japanese randomized phase III trial of adjuvant therapy after surgical resection of biliary cancer (ASCOT trial, JCOG1202) revealed that survivals of patients undergoing adjuvant S-1 treatment for 6 months were significantly better than those of patients with surgery alone (3-yr survival 77.1% vs 67.6%, p=0.008, HR = 0.694), which was presented in ASCO-GI 2022. Adjuvant chemotherapy using S-1 would be the coming standard therapy for resectable ICC. **Keywords:** intrahepatic cholangiocarcinoma; multidisciplinary management; immunotherapy.

S8-3
Role of hypofractionated radiotherapy in multidisciplinary approach for HCC: practical results and mechanism analysis.

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1. **Hypofractionated IGRT is superior to conventional fraction RT.** Image guide hypofractioned IMRT (IG-IMRT) could improve the therapeutic response and confer a potential survival of patients with unresectable but confined intrahepatic hepatocellular carcinoma compared to 3D-CRT with acceptable toxicity. IG-IMRT provided a significantly higher dose of radiation to the tumor, with no increase in mean dose to the whole liver for portal vein tumor thrombi with HCC. The therapeutic dose delivered by IG-IMRT is slightly higher than non-IG-IMRT which was more effective and showed superior short-term survival and local control in HCC patients with lymph node metastases. Hypofractionated IG-IMRT is safe for patients with bone metastases and may achieve pain relief earlier than conventional radiotherapy. Table shows the results of studies comparing survival after hypofractionated IG-IMRT versus convention non-IG-IMRT.

Table. Comparison of survival after hypofractionated IG-IMRT versus conventional non-IG-IMRT

Stage	References	Median OS		P value
		Non-IGRT	IGRT	
BCLC B (confined in liver)	Can J Gastroenterol Hepatol. 2017:6267981.	24.0	44.7	0.009
BCLC C (PVT)	Jpn J Clin Oncol. 2016;46:357–62.	10.5	15.5	0.05
BCLC C (LNM)	Ann Palliat Med. 2019;8:717–27.	9.7	15.3	0.098
BCLC C (lung met.)	Oncotarget. 2016;7(30):48586–48599.	16.7	29.6	<0.001
BCLC C (bone met)	J Cancer. 2019;10(17):4031–4037.	96.7% (pain relief)	91.2% (pain relief)	0.169
BCLC C (Adrenal met)	Clin Transl Oncol. 2017;19:1154–1160.	14.3	42	0.047

2. **Hypofractionated stereotactic body radiotherapy (SBRT) combined with anti-PD-1/PD-L1 antibody in patients with HCC is safety and effective.** SBRT was used in intrahepatic recurrent HCC patients. 25 patients with 32 lesions were treated with SBRT + Sintilimab. SBRT was delivered at a median dose of 54 Gy in 6 fractions and the median follow-up time was 16.9months. The confirmed ORR was 96% with 18 CR and 6 PR. 6-mo and 12-mo PFS rate was 100% and 70%. 12-mo local control rate was 100%. Median PFS and OS were not mature. Treatment-related adverse events (TRAEs) occurred in 14 patients (56%), most common were rash (16%), platelet count decreased (12%), and myositis (8%). Grade 3 TRAEs (myositis) occurred in 1 patient (4%). TRAEs led to discontinuation in 6 pts (24%). SBRT + Sintilimab was tolerable with ORR of 96% in patients with recurrent oroligometastatic HCC. Longer follow up is required to further evaluation.

3. **Hypofractionated RT induced iRhom 2 up-regulation promotes macrophage anti-tumor activity through cGAS/STING signaling.** Hypofractionation brought new questions of dose-fractionation schedule, especially the immunomodulatory effects of radiotherapy changing following dose-fractionation schedules. In the present study, we excluded the direct antitumor effect of radiation by comparing three schedules and found 1 × 10 Gy had significant higher antitumor effect, suggesting a single high dose induced more enhanced anti-tumor immunity compared to the conventionally and moderately fractionation (2 × 8 Gy, 3 × 4.5 Gy). However, in STING deficiency mice, we found no difference in tumor control among the three schedules, suggesting cGAS/STING signaling was the involved regulating pathway leading to different anti-tumor immunity. Further mechanism investigation revealed single high dose irradiation induced up-regulation of iRhom2 in infiltrated macrophage, while iRhom2 promotes cGAS/STING signaling via maintaining stability of STING and facilitating STING trafficking from endoplasmic reticulum to perinuclear microsomes. Meanwhile, positive feedback between iRhom2 and TNFα sharpened the signaling responses, and amplified antitumor immunity induced by cGAS-STING-IFN pathway following single high dose radiation. In conclusion, single high dose irradiation induced iRhom2 up-regulation promotes macrophage anti-tumor activity through cGAS/STING signaling. **Keywords:** image guide hypofractioned IMRT; stereotactic body radiotherapy; hypofractionated RT; cGAS/STING.

Session 9: Conversion therapies for advanced HCC and iCCA

S9-1

Downstaging and resection for unresectable intrahepatic cholangiocarcinoma (ICC): where are we?

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Complete surgical resection remains the only potentially curative treatment for intrahepatic cholangiocarcinoma (ICC). For patients with resectable ICC, capecitabine is recommended as adjuvant therapy following resection, while additional adjuvant and neoadjuvant trials are ongoing to improve the outcomes in this setting. However, most patients with ICC will present with locally advanced disease and would be deemed surgically unresectable due to major vascular involvement (portal and hepatic veins) and other reasons. Current efforts are ongoing to explore the potential effective systemic therapy alone, or in combination with local-regional treatment including Yttrium-90 radioembolization, in an attempt to downstage the tumor to convert it to surgically resectable. While single center experiences have reported the success for downstaging for some of the treatment regimens, no definitive regimens have emerged as the standard downstaging treatment. Moreover, the impact of downstaging on the improvement of overall survival remains to be defined. The author will discuss the rationale, current clinical development, and unanswered questions for the use of downstaging strategy in unresectable ICC. **Keywords:** intrahepatic cholangiocarcinoma; resection; downstage; systemic therapy.

S9-2

Emerging systemic therapies for advanced-stage HCC: finding a cure without resection?

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Since the success of atezo-bev in 2019, novel ICI-TKI combinations, with a hope for an even better efficacy, are being eagerly awaited. However, the hypothetical synergistic effect has not yet been realized. While we are awaiting results of other novel combos, a thourough deep-diving research to dissect the true immune modulatory mechanism of various TKIs is mandatory. Although combinations of double ICIs seem to be promising, breakthrough achievements have not seen so far. ICIs other than anti-CTLCA4, such as anti-LAG3 or anti-TIGIT, are under early trials. Several important breakthrough new modalities in other cancer types, such as antibody-drug conjugates, CAR-T, bispecific antibodies, and RNA cancer vaccines will be discussed. **Keywords:** HCC; systemic therapy; ICI; new modality; breakthough.

S9-3

Conversion therapy in patients with unresectable or intermediate and advanced stage hepatocellular carcinoma (HCC)

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Intermediate or advanced stage HCC account for 2/3 of newly diagnosed HCC in China, who are not ideal candidate for curative treatment. The conversion therapy aims to down-stage or down-size original tumor to facilitate a R0 hepatectomy and achieve a long-term disease free survival. Significant advances in systemic therapy with an improved tumor response and progression-free survival provide increased opportunity for conversion therapy in patients with intermediate or advanced stage HCC. Our initial experience proved the effect of conversion therapy followed by curative hepatectomy. However, there are still many questions remain to be answered. The safety of hepatectomy following systemic therapy, the optimal candidate for conversion therapy, and the value of hepatectomy after tumor response is achieved by systemic therapy will be addressed in this presentation with our initial investigations. **Keywords:** HCC; conversion therapy; hepatectomy.

S9-4

Conversion therapies for advanced-stage HCC: Japan experience

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Novel molecular therapies using targeted drugs and immune checkpoint inhibitors for advanced hepatocellular carcinoma have evolved. Sorafenib and lenvatinib have been commonly used as first-line therapy, followed by recent atezolizumab plus bevacizumab. As a result, the median survival time has gradually improved to over 1.5 years. In addition, the partial and complete response rates are increasing; however, the complete radiological response does not always mean a complete pathological response and a permanent cure for the disease. To resolve this, conversion surgery has developed. Conversion therapy is defined as the status that down-stages unresectable HCC to resectable HCC. It also includes the status that unresectable extrahepatic metastases become resectable or stable. Lenvatinib was approved as a first-line tyrosine kinase inhibitor in Japan, and its use spread worldwide in 2018. Lenvatinib can be the most suitable drug due to its high response rate in the present era. A recent large cohort study using lenvatinib had a conversion rate of 8.4% and an estimated median disease-specific survival time of >80% at three years. In addition, conversion to curative resection was an independent predictive factor for better disease-specific survival compared with lenvatinib monotherapy. A Japanese multicenter observational study of surgical resection results following lenvatinib therapy for initially unresectable HCC was reported in ASCO-GI in 2022. In this presentation, we will summarize the current status of conversion surgery in Japan for advanced HCC after molecular therapies focusing on long-term survival and disease cure. Furthermore, we should discuss promising drugs and the timing for conversion surgery. **Keywords:** advanced HCC; conversion surgery; conversion therapy; Japan.

State-of-the-Art Lecture 4

SL4-1

Sequential therapy for HCC after failure of atezolizumab plus bevacizumab

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Atezolizumab plus bevacizumab has been demonstrated marked superiority to sorafenib and been the first-line therapy from 2020. It is expected that 70–80% of patients who have started first-line combination therapy were eligible for second-line, thus, the second-line therapy is critical. This editorial discusses the choice of sequential therapy after the combination failure. The current expectation is sorafenib and lenvatinib will become second-line treatments, and regorafenib, etc. will become third-line. WNT/β-catenin mutations, which activate β-catenin, are found in

20–30% of HCCs. The immune classification of HCCs categorizes WNT/β-catenin mutations into the immune exclusion or immune cold subclasses. As with other carcinomas (e.g., renal, bladder, and ovarian), HCCs with elevated β-catenin protein levels also show less intratumoral T cell infiltration. However, FGFR4 expression is higher in the population of tumors with WNT/β-catenin-activating mutations, and there is a positive correlation between β-catenin mutations and FGFR4 expression. It is well known that lenvatinib has a potent inhibitory effect on FGFR4. Yamauchi et al. conducted a study of 40 patients with HCC and reported lenvatinib achieved higher response rate (81%) and longer PFS (5.5 months) in tumors with high than without FGFR4 expression (31%, 2.7 months). Thus, even in patients who do not respond well to atezolizumab plus bevacizumab due to β-catenin-activating mutations, subsequent treatment with lenvatinib would still provide better results. Lenvatinib has been demonstrated at our hospital extremely high efficacy in patients who had progressed on previous therapy with a PD-(L)1. Specifically, lenvatinib following failure of PD-1/PD-L1 improved PFS (10 months), OS (15.8 months) (from the start of Lenvatinib), ORR (55.6%) and DCR (86.1%). OS since initiation of ICI therapy was 29.8 months, which is much longer than lenvatinib alone as first-line therapy. Now, lenvatinib is probably the most used second-line treatment and is expected to provide higher response rate PFS than other TKIs. Actually, in our study, lenvatinib treatment after PD-1/PD-L1 antibody failure provided much better outcomes than lenvatinib or nivolumab alone as the first-line therapy. However, which sequence is the best will be disclosed in real-world settings. Through such studies, it will be more important to clarify the therapy sequential after failure of atezolizumab plus bevacizumab combination therapy. **Keywords:** HCC; second-line therapy; WNT/β-catenin mutation; FGFR4; lenvatinib.

Session 10: Immuno-oncology in liver cancer: research and clinical advancement

S10-1

Locoregional treatment for HCC in the era of immunotherapy: hype or hope?

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Newer systemic therapies, atezolzumba plus bevacizumab, and durvalumab plus tremelimumab, are expected to change traditional treatment strategies due to improved survival and reduced adverse events regimens. Systemic therapy has been recommended for patients who are expected to require repeated locoregional treatment, even for HCC localized in the liver, and for those who do not respond to repeated locoregional treatment. However, it is estimated that the indication and timing of these recommendations will change due to the advent of immunotherapeutic agents that are more effective and show fewer side effects. The advent of immunotherapeutic agents for HCC raises concerns not only about the indication of the first treatment but also about how to do secondary of subsequent treatment for immunotherapeutic responders. It is necessary to evaluate the strategy of applying locoregional treatment after immunotherapy or applying combination treatment with locoregional and systemic immunotherapy. However, there is no study comparing the new first-line treatment of atezolizumab plus bevacizumab with a combination therapy of locoregional treatment. We are waiting for the results of an ongoing phase 3 randomized controlled trial (RCT) of transarterial chemoembolization (TACE) plus immunotherapy. Although the phase 3 RCT of combination therapy with TACE and tyrosine kinase inhibitors (sorafenib, brivanib) for patients with advanced HCC in the past has failed, there is hope for the combination therapy with TACE and immunotherapy. Among patients with advanced HCC, if the liver function is grade B or the risk of bleeding is high, it may be challenging to apply immunotherapy, so local treatment should be carefully considered. Although the currently available immunotherapeutic agents have improved overall survival and/or progression-free survival compared to the past, they are not satisfactory compared to the outcome of other cancers. As there are currently no biomarkers, it is considered that combination treatment or sequential treatment of locoregional treatment is necessary to improve the outcomes of patients with HCC. **Keywords:** HCC; locoregional treatment; immunotherapy; combination therapy.

S10-2

Immuno-oncological perspective of liver-directed treatment

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The intrahepatic balance between immunity and tolerance for the antigen influx from the gastrointestinal tract and systemic circulation may link to the compromised immune-surveillance and the developed hepatocellular carcinoma (HCC). Non-surgical liver-directed treatments to HCC, including RFA, TACE, TARE, and radiotherapy (RT), are the mainstays for patients with early-/intermediate-stage disease. Although several immunotherapeutic drugs especially checkpoint inhibitors have been approved for their uses in advanced HCC with the improved survivals, the combination of liver-directed treatments and immunotherapy is a potential strategy of hope as a curative setting. The rationale to combine immunotherapy with locoregional therapy is based on the phenomenon of ablated, embolized, or irradiated tumor may activate the release of tumor-associated neoantigens and allow the recognition of immune cells in the tumor microenvironment. Preclinical experiments and clinical pilot studies demonstrated the activated immune cells (T cells, NK cells, macrophages, etc.) and molecules (PD-1, PD-L1, TIM-3) from tumor/serum associated with improved prognosis. Liver-directed modalities, such as RT, can increase the expression of PD-L1 on the surface of tumor and immunosuppressive myeloid cells, as well as the expression of TIGIT (a co-inhibitory receptor expressed on CD8+ T cells), NK cells, Tregs, and T follicular helper cells. With the success of adding targeted drugs (anti-angiogenesis) to checkpoint inhibitor in

advanced HCC, such a combination with locoregional therapy may be logically strengthened. Besides, PD1/PD-L1 blockade can reverse the exhausting T-cell pathway induced by local treatment. However, the delicate mechanism of the combinational synergism by immunotherapy can be confounded by the sequencing, dosing, and microenvironmental issues with the use of locoregional treatments. Ongoing randomized trials, such as DEMAND (TACE plus atezolizumab/bevacizumab), though mostly in a limited scale, may provide answers to some of these questions and help settle the roles of combining liver-directed treatments with immunotherapy in patients with early, intermediate, or advanced HCC. **Keywords:** hepatocellular carcinoma; liver-directed treatment; locoregional therapy; immunotherapy.

S10-3

Immunotherapy in non-alcoholic steatohepatitis related HCC

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Atezolizumab plus bevacizumab has been demonstrated significant improvement in OS and PFS in the IMbrave150 trial and as the new first-line standard of care for unresectable HCC. Other combinations (HIMALAYA trial, COSMIC-312) also showed similar efficacy or are under investigation (LEAP-002, Checkmate 9DW and others). An important ongoing debate discusses that whether the underlying etiology of HCC has an impact on the response to systemic treatment, which fueled by post-hoc subgroup analysis of the IMbrave150 trial with no difference for OS and PFS between atezolizumab plus bevacizumab and sorafenib in patients with non-viral etiology. This observation led to the hypothesis of an impaired efficacy of immune therapy in NAFLD/NASH associated HCC, which presents a relevant proportion of non-viral etiology. It should be kept in mind, though, that subgroup analyses at best are hypothesis generating and not proving differential efficacy. This can be highlighted by the efficacy of sorafenib in different trials. SHARP trial showed a median OS for HBV-HCC and HCV-HCC of 9.7 and 14 months, conversely, the data in the sorafenib arm of the IMbrave150 trial were almost identical (12.4 vs 12.6 months). Similar discrepancies have also been observed: IMbrave150 trial showed no difference as above, but HIMALAYA trial did show significant difference for tremelimumab plus durvalumab over sorafenib with HR of 0.74, interestingly, no difference for HCV-HCC. As non-viral and viral etiologies were stratification factors in HIMALAYA trial, this is a relevant observation; however, the treatment regimens were different, anti-PD-L1 plus anti-VEGF on one hand and plus anti-CTLA4 on the other. Finally, it should be mentioned that the lack of difference in the two arms in IMbrave150 stems from strongly over-performing sorafenib (mOS 18.1 vs 13.4 months) and not under-performing atezolizumab plus bevacizumab (mOS 17 vs 19.2 months), when comparing efficacy in non-viral patients with all. In summary, post-hoc analyses are prone to bias and should be treated carefully: this includes imbalances in the prognostic factors (AFP, tumor burden, EHS, MVI, etc.), a lack of control of post progression treatment affecting OS, no control of liver function during and post treatment, etc. Moreover, the non-viral subgroup is ill defined, comprising NASH, alcohol, and other etiologies. Finally, only prospective RCTs may prove or falsify the hypothesis. **Keywords:** non-alcoholic steatohepatitis related HCC; immunotherapy; impaired efficacy of immune therapy.

Session 11: Optimal managements for intermediate HCC

S11-1

Optimal management for intermediate HCC Treatment strategy for HCC patients with BCLC stage B in Kurume university hospital

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Liver cancer is the third leading cause of cancer-related death and ranks as the sixth most common neoplasm in 2018. Hepatocellular carcinoma (HCC) accounts for approximately 75%–85% of all primary liver cancers. It is estimated that 72% of cases occur in Asia, 10% in Europe, 7.8% in Africa, 5.1% in North America, 4.6% in Latin America and 0.5% in Oceania. Treatment for HCC is decided according to the staging system with information on tumor burden and liver function. The Barcelona Clinic Liver Cancer (BCLC) staging system, which was first introduced in 1999, is highly evidenced-based and the most commonly used worldwide. Although the intermediate-stage (BCLC stage B) includes the largest number and heterogeneous HCC patients, the recommended treatment option was transarterial chemoembolization (TACE) only for a long time. In 2022, the last updated BCLC staging system recommended three treatment options, liver transplantation, TACE and systemic therapy for HCC patients with BCLC stage B. However, recent progress in radical treatments such as hepatic resection, radiation therapy, and percutaneous therapy has made it possible to treat selected patients with BCLC stage B HCC. Radical treatments are expected to prolong survival time. To-date, TACE has also progressed. In addition to conventional TACE, Balloon-occluded TACE and Drug-eluting beads TACE are available. These new modalities of TACE will improve therapeutic efficacy and reduce adverse events. In addition, many molecular targeted agents and immune checkpoint inhibitors are now available for advanced HCC patients with Child-Pugh class A worldwide. Treatment of HCC with BCLC stage B is becoming complicated. Under these circumstances, many hepatologists and oncologists in Asian countries have made efforts to adopt the principles of the BCLC staging system; in contrast, they are also independently making efforts to expand the indications of each treatment and to combination therapies as well as alternative therapies for better outcomes. In this presentation, I will introduce our treatment strategy for HCC with BCLC stage B. **Keywords:** Intermediate HCC; BCLC stage B; treatment strategy.

S11-2

Position of curative therapies for intermediate-stage HCC

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Patients diagnosed with intermediate-stage hepatocellular carcinoma (HCC) in Barcelona Clinic Liver Cancer (BCLC) staging system, encompass a broad clinical population. Liver resection for intermediate-stage HCC remains controversial. The progress of drug therapy, such as molecular targeted therapy and immune checkpoint inhibitor, is repositioning surgical treatment for advanced HCC. The proper combination of drug therapy and surgery may improve treatment outcomes for patients who are unsuitable for resection, i.e., resectable but have a poor prognosis, but proper selection of subjects is unclear. The purpose of this study is to discuss future treatment strategies and indication based on past treatment results of intermediate-stage HCC. Intermediate-stage HCC is an oncologically heterogeneous population, with some cases being resectable and others unresectable. Furthermore, even among resectable cases, some populations have a better prognosis with liver resection, while others have a worse prognosis equivalent to TACE. We developed a risk score to identify the poor prognosis group based on a study of 382 resected cases of beyond BCLC at the Hiroshima Surgical study group of Clinical Oncology Hiroshima (HiSCO). This risk score including a high a-fetoprotein level, macrovascular invasion, and high total tumor burden, well stratified prognosis of beyond BCLC resection cases. Several systems for subdividing Intermediate-stage HCC have been reported. When divided into 3 groups by Kinki-criteria including tumor burden, liver resection significantly outperformed TACE in the B1 group (lower tumor burden) but was comparable to TACE in the B2 group (higher tumor burden). The proper selection criteria of combination of drug therapy and surgery remains undefined. Tumor markers and tumor burden may be criteria for selecting combination therapy. **Keywords:** Intermediate HCC; curative therapy; treatment strategy.

S11-3

Nonsurgical management of intermediate-stage HCC

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Intermediate-stage hepatocellular carcinoma comprised a very heterogeneous populations, these patients might not benefit from surgical treatments. Locoregional interventional therapies play a leading part in the management of HCC, and it is estimated that 50–60% of patients with HCC might receive those treatments in their lifespan. TACE has been recommended therapy for those patients. Median overall survival (mOS) were about 26-30 months in those patients with intermediate-stage HCC treated with TACE. However, in the past two decades, whether with the application of novel materials of embolization, such as drug-eluting beads or combination with molecular targeted drug, no improvement of OS was observed. How to improve outcomes of intermediate-stage HCC is one of the most important problems. The prognosis of HCC was affected by tumor burden, preserved liver function, performance status, etiology, etc. Even in recommended TACE candidates, OS is heterogenous. Scores proposed for patient selection and/or re- treatment with TACE have not yet been universally adopted. An easy-to-use individual method to predict the prognosis with high performance is needed. Systemic treatments with molecular and immune therapies have dramatically changed the management of cancer. the positive results of a phase III trial demonstrating superior results for atezolizumab plus bevacizumab versus sorafenib for advanced HCC heralded a new era of combination therapies and set a new benchmark for survival benefits. Currently, RCTs are in progress with immunotherapy alone or combined with molecular therapy, either in combination with or directly challenging chemoembolization in intermediate- stage disease. Results of these trials will be public within the next 5 to 10 years. Although several therapeutic alternative transarterial devices (such as TARE) have challenged TACE, none of them has been able to substantially improve the standard of care established decades ago. However, we still admit there is a long way to go to improve OS of patients with intermediated-stage HCC. **Keywords:** Intermediate HCC; nonsurgical management; treatment strategy.

S11-4

The plasticity of clonal evolution in intrahepatic metastasis based on single cell sequencing

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Background: Hepatocellular carcinoma (HCC) patients often present multiple intrahepatic tumors of which the biological behavior with intrahepatic metastasis is more invasive, and patients are more prone to recurrence and poor prognosis after surgery. In addition, due to unclear mechanism and poor therapeutic effect, the mechanism of intrahepatic metastasis has become a hot and difficult research topic. The heterogeneity of tumor microenvironment is important for inducing metastasis of HCC. Therefore, in this study, the heterogeneity of tumor microenvironment in multifocal HCC with intrahepatic metastasis was evaluated at bulk transcriptome level and single cell transcriptome level, and the metastasis mechanism of HCC was explored. **Methods:** A total of 2 HCC patients with intrahepatic metastases were included, with 2 primary tumor samples and 3 metastatic tumor samples. Patients must ≥18 years with radiographic and histological or cytological diagnosis of HCC. Each patient has about 2-3 separate tumors. All five samples were sequenced using 10× Genomics sequencing platform and routine single-cell data analysis was performed. **Results:** Primary tumor and intrahepatic metastases tumor samples share all immune and stromal cell types. Malignant epithelial cells with intrahepatic metastases tumor samples are more heterogeneous than those with primary tumor samples, and the evolutionary trajectory of malignant epithelial cells with primary tumor samples to malignant epithelial cells with metastases tumor samples can be

obtained based on cell lineage analysis. By comparing the nonepithelial cells in the primary and metastatic tumor samples, CD8+T cells were found to be highly infiltrated in the metastatic samples, and the proportion of infiltration of different CD8+T cell subtypes were significantly different from that in the primary tumor samples, suggesting that CD8+T cells play an important role in the process of intrahepatic metastasis. Besides, exhausted CD8+T cells with high expression of multiple immune checkpoint molecules were highly enriched in intrahepatic metastases samples, and the interaction between exhausted CD8+T cells and LAMP3+DC increased in the metastases samples. **Conclusion:** Exhausted CD8+T cells and LAMP3+ DC play a significant role in the intrahepatic metastasis of HCC. It is necessary to conduct large-scale research and experimental verification in the future to further clarify the key genes that nonepithelial cell play a role in the intrahepatic clonal evolution of multifocal HCC. **Keywords:** hepatocellular carcinoma; intrahepatic metastasis; clonal evolution; mechanism; CD8+T cell.

Session 12: Young scientists symposium

S12-1

Genome-scale screen for synthetic drivers of T cell proliferation

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Genetic engineering of patient T cells for adoptive cell therapies has revolutionized the treatment of several cancer types, most notably B-cell malignancies. However, further improvements to engineered T cells are needed to increase the durability of and response rate to these therapies. While CRISPR-based loss-of-function screens have shown promise for high-throughput identification of genes that modulate T cell response, these methods have been limited thus far to negative regulators of T cell functions and raise safety concerns due to the permanent nature of genome modification. Here we identify positive T cell regulators via genome-scale overexpression of barcoded libraries of human open reading frames (ORFs). ORF library overexpression enables the identification of modulator genes that may not be expressed by T cells or whose expression is limited only to a certain T cell subsets or states. We also developed a method for directly assigning the identity of overexpressed ORFs to single cells, thus enabling high dimensional quantification of ORF overexpression on the transcriptome and surface proteome in engineered T cells. We show that top-ranked hits from the genome-scale ORF screen increase primary human CD4+ and CD8+ T cell proliferation, activation and secretion of key cytokines. One of these top-ranked ORFs, lymphotoxin beta receptor (LTBR), is typically expressed in a subset of myeloid cells but absent in lymphocytes. When expressed in T cells, we find that LTBR increases their resistance to apoptosis and boosts the secretion of proinflammatory cytokines. For LTBR and several other top-ranked genes, we show that co-expression improves antigen-specific chimeric antigen receptor (CAR) T cell response. Finally, we demonstrate that top-ranked ORFs from CD4+ and CD8+ αβ T cells also improve antigen-specific responses in γδ T cells which recognize diverse cancer types regardless of the patient's major histocompatibility complex (MHC) haplotype, potentially enabling future population-wide, cancer-agnostic therapy. **Keywords:** engineered T cell; positive T cell regulator; genome-scale; T cell proliferation.

S12-2

Mutational signatures and processes in intrahepatic cholangiocarcinoma

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Intrahepatic cholangiocarcinoma (iCCA) accounts for 10% of primary liver cancers (PLCs), but 20% of overall PLC-related mortality. The incidence have significantly increased over decades. Alarmingly, iCCA mortality rates are also increasing, superseding most other cancer types[1], increased by 90% between 2002–2012 in Denmark. These facts implicate iCCA as a growing socioeconomic and healthcare burden. iCCA is often incidentally diagnosed after radiological workup prompted by detection of abnormal circulating liver function markers, but non-invasive biomarkers are lacking. Many iCCAs are sporadic with no obvious risk factor, impeding monitoring of 'at-risk' demographics for iCCA. 12-40%[2] of patients diagnosed with localized disease, and surgical resection is the primary management with curative intent, but subclinical metastases is the main cause of failure, and 54-73% of patients relapse within 5 years even achieved R0 surgical margin. The high recurrence rate argues novel molecular tools are required to predict subclinical relapse. Pervasive tumor heterogeneity, inherent at the anatomical, histopathological, cellular and genomic levels, is arguably key obstacles to elucidate novel targeted therapies in iCCA[3]. The initiation of these cancers might involve common mutagenic processes. However, while many risk factors lead to random genomic damage, others generate damage in specific sequence contexts throughout the genome. The hepatobiliary system is exposed to a variety of exogenous genotoxins. Diverse insults[4] require the epithelial liver cells to efficiently repair DNA damage and correctly replicate DNA during division in often challenging microenvironments (surrounding desmoplasia, inflamed and cirrhotic liver parenchyma). Analysis of this catalogue of somatic alterations and the mutations genomic sequence is possible to deduce the malignant processes. These mutational signatures emphasize the diversity of the mechanistic processes active in iCCA, which may explain the high tumor heterogeneity. Integrated genomics approaches have been used to classify iCCA patients based on prognosis[5], emphasizing a plethora of dysregulated oncogenic signaling pathways. Indeed, iCCA can be classified solely based on mutations in key driver-genes (*TP53, KRAS* and *IDH1/2*) that elucidate unique mutational signatures, structural variants and epigenomic alterations compared to wildtype tumors[6]. Our approach has emphasized specific onco-genetic mechanisms

distinct in each patient subset with potential unique drug responses (RNA synthesis inhibition in *IDH*-mutant, microtubule modulator in *KRAS*-mutant, topoisomerase inhibition in *TP53*-mutant and mTOR inhibitors specific in wildtype tumors. Interestingly, the `wildtype´ patient subset was enriched in Fibroblast Growth Factor Receptor 2 (FGFR2) fusions. Besides, isocitrate dehydrogenase (IDH) mutations, epigenetic regulation plays a pivotal role in the general oncogenesis of iCCA with a significantly deregulated DNA methylation landscape and prevalent mutations in the SWI/SNF complex, histone lysine demethylases (KDMs) and lysine methyltransferases (KMTs). **Keywords:** intrahepatic cholangiocarcinoma; mutational signature; biomarker; tumor heterogeneity; integrated genomics.

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S12-3

B cell plasticity and diversity impact on liver cancer treatment

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Background: B cells constitute a major component of tumor-infiltrating leukocytes. However, the influence of these cells on malignancy is currently under debate, reflecting the heterogeneity of B cell subsets in tumors. With recent advances, it becomes apparent that this debate includes not only the evaluation of B cells themselves, but also the underlying immune microenvironment network, which scripts the highly heterogeneous B cell populations in tumors and directs the roles of those sub-populations in disease progression and clinical treatment. **Methods:** In our studies, by using hepatocellular carcinoma (HCC) as a model system, we have identified several types of protumorigenic B cell subsets and elucidated their regulating roles during cancer progression. **Results:** First, $CXCR3^+$ B cells migrate to the inflamed stroma of HCC; after interacting with the T_{FH}-like cells or $CXCL10^+$ macrophages, these cells differentiate into IgG^+ plasma cells and subsequently promote formation of M2b macrophages and dismantle immunotherapeutic efficacy of epigenetic reprogramming. Second, in advanced-stage HCC, $PD\text{-}1^{hi}$ identifies a novel regulatory B-cell population that exhibits a unique $CD5^{hi}CD27^{hi/+}CD38^{dim}$ phenotype; upon encountering $PD\text{-}L1^+$ cells or undergoing PD-1 triggering, $PD\text{-}1^{hi}$ B cells suppress tumor-specific T-cell immunity and promote cancer growth via IL-10 signals. Third, environmental semimature dendritic cells can operate in a CD95L-dependent pathway to generate $Fc\gamma RII^{low/-}$ activated B cells; activated $Fc\gamma RII^{low/-}$ B cells from without external stimulation suppress autologous tumor-specific cytotoxic T-cell immunity via IL-10 signals. **Conclusion:** Studying the composition and functions of infiltrating B cells in human tumors may help us better understand the actual roles of B cells in tumor pathogenesis. Moreover, we also focus on whether B cells, as well as their interacting network, can serve as therapeutic targets and suggest that HCC patients may be treated with epigenetic reprogramming in combination with B cell depletion, FcγR blockade, or Syk-Btk inhibition. Studying the mechanisms that can selectively modulate functional activities of inflammatory stroma cells can provide a novel strategy for anticancer therapy. **Keywords:** hepatocellular carcinoma; protumorigenic B cell; B cell regulating.

S12-4

Genomic characterization and evolution in liver cancer

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Background: Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) are the two most common liver cancer. We aimed to systematically define the genomic alterations in Chinese patients with HCC or ICC. **Methods/Results:** We identified Signature 22 (aristolochic acid) and 24 (aflatoxin) widely exist in both HCC and ICC from China, which were not common in those from TCGA or ICGC. In addition to mutations in well-known cancer genes, we identified some new drivers, such as *WNK2* in HCC and *SAV1* in ICC. More importantly, we revealed the association of some driver gene mutation subtypes, such as *KRAS* and *BRAF*, with prognosis and targeted therapy response in a large ICC cohort, which can help to guide precise treatment for patients with ICC. In addition to primary liver cancer, we sought to infer the tumor subclonal architectures and track the genomic evolution from primary tumor to relapsed tumor. We identified two recurrence patterns during HCC early recurrence: de novo recurrence, which developed genetically independently of the primary tumor and carried different HCC drivers, and ancestral recurrence, which was clonally related to the primary tumor and progressed more rapidly than de novo recurrence. We also demonstrated spatiotemporal heterogeneity and polyclonal, monophyletic dissemination in HCC ancestral recurrence. In ICC, we revealed polyclonal seeding of ICC intrahepatic dissemination during relapse, and identified *SLIT2* as a driver of tumor dissemination and tumor-associated neutrophil infiltration. **Conclusion:** We systematically defined the genomic alterations and the

evolutionary trajectory in liver cancer from China. **Keywords:** hepatocellular carcinoma; intrahepatic cholangiocarcinoma; genomic alteration; subclonal architectures; genomic evolution.

S12-5

Dissecting tumor ecosystem in early-relapse hepatocellular carcinoma by single-cell RNA sequencing

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Background: Liver cancer is one of the highest causes of cancer-related deaths worldwide. Hepatocellular carcinoma (HCC) is the predominant form of liver cancer, accounting for 75%–85%, for which surgical resection represents the most effective treatment, with curative potential. However, the high incidence of tumor relapse (50%–70% 5 years after surgery) has hindered improved survival. Furthermore, early recurrence within 2 years after surgery accounts for 70% of relapsed HCC cases, is rarely treatable, and has been associated with poor survival. The molecular mechanisms associated with rapid postoperative recurrence remain poorly understood. A thorough exploration of tumor relapse could enhance our understanding of the mechanisms associated with tumor development and progression and help discover more effective therapeutic strategies for HCC. Tumors are complex ecosystems, defined by spatiotemporal interactions among heterogeneous cell types, including malignant, immune, and stromal cells. Understanding the interplay of cell types is essential to understand tumor development, prognosis, and treatment. Several studies have profiled the single-cell landscape of immune cells and tumor cells' heterogeneity in primary HCC. However, a comprehensive depiction of the ecosystem and immune phenotypes in early-relapse HCC at the single-cell resolution level remains lacking. **Methods:** We dissected the tumor immune microenvironment by examining 16,498 full-length single-cell transcriptomes from 18 primary or early-relapse HCC patients. We observed reduced levels of T-regulatory cells (Tregs) and increased fractions of dendritic cells (DCs) and CD8 + T cells in the immune component of early-relapse tumors (RT), compared with those in primary tumors (PT). **Results:** Interestingly, CD8 + T cells in RT, characterized by overexpression of KLRB1 (CD161), primarily presented an innate-like, low-cytotoxic, and low-clonal-expansion state, with the low expression of co-stimulatory and checkpoint molecules. Patients with more CD161+ CD8 + T cells showed a worse prognosis. We validated these findings in two additional cohorts of paired PT and RT samples, using scRNA-seq or immunohistochemical (IHC) staining. **Conclusion:** Our analysis showed that malignant cells in RT have higher immune evasion characteristics, which potentially inhibit the capacity of DCs to activate CD8 + T cells. Our data shed light on the unique aspects of the altered immune response associated with HCC relapse, which might guide the development of rational immunotherapies to benefit a wide range of patients. **Keywords:** hepatocellular carcinoma; tumor immune microenvironment; relapse tumor; KLRB1.

Session 13: Abstract Oral Presentation

S13-1

Adjuvant lenvatinib after radical resection in patients with hepatocellular carcinoma (HCC): preliminary analysis of a prospective, multi-center, single-arm study

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Aims: Chinese guidelines recommend surgical resection for patients with China Liver Cancer (CNLC) stage Ia–IIIa HCC (equal to BCLC stage A/B and selected patients with BCLC stage C). Over 60% HCC patients in China have already been CNLC stage II/III (BCLC B/C) of whom most are not suitable for surgical treatment, however, some with CNLC stage IIb and IIIa still benefit from surgery, but have a high recurrence risk and poor overall survival (OS). This study was conducted to evaluate the efficacy and safety of adjuvant lenvatinib in patients with high risk of disease recurrence. **Methods:** In this multi-center, single-arm, prospective trial (NCT04227808), patients underwent radical resection for CNLC Stage IIb/IIIa HCC within 4-6 weeks were eligible and received treatment of lenvatinib (8 or 12 mg/day) until disease recurrence, intolerable toxicity, or death. Because lenvatinib was used as an adjuvant therapy, more aggressive dose adjustments were allowed, including dose reduction and discontinuation, to lower the incidence of severe adverse reactions. The primary endpoint was 1-year recurrence-free survival (RFS) and secondary endpoints included OS and safety. Planned enrollment was 50 patients and here we report a preliminary analysis. **Results:** In total, 51 patients were enrolled from Mar 2020 to Dec 2021 and 48 patients were eligible and had at least one set of follow-up data at the cut-off date (July 22, 2022) and were included in the present analysis. Of these 48 patients, the median age was 56 years, 83.3% were male. 75% were HBV positive (8 of them were also HCV positive) and 25% were non-viral. Seven patients (14.6%) had CNLC stage IIb and 41(85.4%) had CNLC stage IIIa HCC. The median follow-up time is 14.6 months. 22.9% experienced dose reduction or delay. The 1-year RFS rate was 61%, the median RFS was 19.33 months (95% CI: 11.3–27.4). Four patients died after tumor recurrence. In total, 91.7% of patients experienced ≥1 treatment-related adverse event (AE) of any Grade and 14.6% had Grade 3 treatment-related AEs. No treatment-related deaths occurred. **Conclusions:** Adjuvant lenvatinib was well tolerated in patients with CNLC stage IIb/IIIa HCC after R0 resection. The median RFS was longer than our historical data (mRFS=9.03 months), and these findings warrant further investigation in a controlled study. **Keywords:** hepatocellular carcinoma; CNLC stage IIb/IIIa; adjuvant therapy; R0 resection; lenvatinib.

S13-2

The impact of transfusion-free curative hepatectomy on the overall prognostication in patients with HCC

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Aims: Hepatectomy is a well-established curative treatment for hepatocellular carcinoma (HCC). However, blood transfusion (BT) sometimes cannot be avoided. This study examines the impact of BT for operated HCC. **Methods:** This is a single centre retrospective analysis from January 2010 to December 2019. Data of HCC patients undergoing curative hepatectomy was reviewed. Patients who had received perioperative BT were compared with those without. A propensity score matching analysis (PSM) was performed. The groups were compared. **Results:** Totally 846 patients were included in the study and overall transfusion rate was 14.9%. A PSM using age, gender, preoperative hemoglobin level, and the staging of the underlying HCC were matched in a ratio of 2:1 (Non-BT:BT). There were more patients in the BT-PSM with more underlying comorbidities (p=0.043), and the ICG at 15 minutes was higher (p=0.011). The AFP level was higher in the Non-BT-PSM group (p=0.045). Both groups had similar extent and modality of the operation. More patients received pringle maneuver in the BT-PSM group during the operation(p<0.001). There were more patients in BT-PSM group suffered from overall complications (p<0.001) and major complications (p<0.001); They also had a longer hospital stay (12 days, p=0.009) and more 90-day mortality (6.8%, p=0.004). Both groups had a similar disease-free survival, but non-BTPSM group had a better overall survival (5-year survival rate, 57.3% vs 46.6%; p=0.034). Multivariate analysis suggested blood transfusion, higher AFP, and presence of micro- and macrovascular invasion were poor prognostic factors for overall survival. **Conclusions:** A transfusion-free hepatectomy for HCC might result in better long-term oncological outcome. **Keywords:** hepatocellular carcinoma; curative hepatectomy; impact of blood transfusion; transfusion-free hepatectomy.

S13-3

Expression of toll-like receptor 7 and its downstream interferon lambda 1 and nuclear factor kappa B in hepatitis C virus-related hepatocellular carcinoma: relation to tumor progression

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Aims: Toll-like receptor 7 (TLR7) is an intracellular pattern recognition receptor of the innate immune system primarily senses single-stranded RNAs including HCV virus. Stimulation of TLR7 induces type III interferon(IFNL) and activates nuclear factor kappa B (NF-κB) to stimulate proinflammatory genes. This work aims to study the expression of TLR7 and its downstream effectors IFNL1 and NF-κB in HCV-related HCC and their relation to tumor progression. **Methods:** Twenty patients with HCV-related cirrhosis and HCC who underwent tumor resection surgery were included. **Results:** Positive cytoplasmic immunostaining for TLR7 and IFNL1 was detectable in the tumor cells of 60% of HCCs and 95% the surrounding non-neoplastic liver tissues. Positive nuclear immunostaining of NF-κB was detectable in the tumor cells of all HCCs and in 70% of the surrounding non-neoplastic liver tissues. The TLR7 and IFNL1 staining scores showed significant decreases while NF-κB showed significant increase in HCC tissues compared with the surrounding non-neoplastic liver tissues ($P = 0.012$, $P = 0.002$, and $P = 0.005$ respectively). The intratumoral TLR7 and IFNL1 staining scores were positively correlated ($r = 0.820$, $P < 0.001$) and both showed inverse correlations with serum HCV RNA levels, AFP levels, HCC maximum diameter, BCLC stage, and HCC histological grade ($P < 0.05$). The NF-κB expression in HCCs was positively correlated with ALT levels, HCC maximum diameter, and HCC histological grade but not with TLR7 and IFNL1 expression ($P < 0.05$). There were no significant correlations between TLR7, IFNL1, and NF-κB expression in HCCs and Child-Pugh score, MELDNa score and the histological changes in the surrounding non-neoplastic tissues ($P > 0.05$). **Conclusions:** Down-regulation of TLR7 signaling may play an important role in the development and progression of HCV-related HCC mainly through impairment of IFNL1 production but was not enough to suppress the NF-κB pathway, which may be activated by other non-TLR-7-mediated pathways. **Keywords:** hepatitis C virus-related hepatocellular carcinoma; tumor progression; toll-like receptor 7; interferon lambda 1; nuclear factor kappa B.

	Assess/Classification criteria
The severity of liver disease	Child-Pugh classification and MELDNa score
HCC	BCLC staging system
Tumor	Edmondson-Steiner criteria
surrounding non-neoplastic liver tissue	METAVIR scoring system and the steatosis grade
Immunohistochemical examination	antibodies against TLR7, IFNL1, and NF-κB/p65 and the staining intensity was scored semi-quantitatively as a percentage of the total liver tissue area

S13-4

A novel Peng's test in reducing bile leakage after partial hepatectomy for hepatocellular carcinoma: from an animal study to a clinical cohort propensity score matching comparative study

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Aims: Bile leakage (BL) is a common complication of partial hepatectomy for hepatocellular carcinoma (HCC). However, various intraoperative approaches to detect BL have not been widely accepted owing to uncertainty in their treatment effectiveness and complexity of use. The purpose of this study was to propose a new

method for intraoperative bile leakage detection, in order to reduce the incidence of postoperative bile leakage. **Methods:** A novel BL-detection approach (Peng's test) was developed in a swine model to determine the pressures generated in the gallbladder and common bile duct (CBD) during the test. A comparative study was then conducted on a prospective cohort of patients using Peng's test versus a retrospective historical cohort patient group using the White Gauze test in partial hepatectomy for HCC. Propensity score matching (PSM) was performed in a 1:1 ratio to balance confounding factors. **Results:** The maximum pressures with methylene blue injection in the gallbladder and CBD without Pringle's maneuver in the four swines were 103.8 ± 11.8 and 42.3 ± 6.1, respectively. After Pringle's maneuver, 32.0 ± 6.8 mL methylene blue injection led to a maximum pressure in the CBD of 85.3 ± 9.5 cmH2O. The pressures in CBD were 25.8 ± 3.3 and 86.0 ± 9.9 cmH2O when BL appeared at small bile ducts and around the ligation sites, respectively. Of the 206 patients enrolled in the historical control group, 31 (15.0%) developed BL, while of the 54 patients in the study group, only 1 developed grade A BL. The number of BL detected by the routine white gauze test in the control group was significantly lower than that in the study group ($Z = -3.002$, $P = 0.003$). After PSM, the incidence of BL in the control group and grade B/C BL was 20.4% and 11.1%, respectively. The corresponding incidences in the study group were 1.9% ($\chi 2 = 7.594$, $P = 0.006$) and 0% ($P = 0.027$), respectively. The length of hospital stay in the study group was significantly reduced ($Z = -6.048$, $P < 0.001$). **Conclusions:** Peng's test for intraoperative BL detection is safe and effective in reducing BL after hepatectomy. **Keywords:** hepatocellular carcinoma; Peng's test; bile leakage after partial hepatectomy; bile leakage detection approach.

S13-5

Efficacy and safety of donafenib combined with TACE and anti-PD-1 antibody in the treatment of unresectable hepatocellular carcinoma: a retrospective study

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Aims: For unresectable hepatocellular carcinoma (uHCC), combination therapy with TACE and molecular targeted agents has been reported to have higher antitumor efficacy than respective monotherapies. Donafenib is a novel oral multi-target anti-angiogenesis and its superiority over sorafenib has been demonstrated as first-line treatment for uHCC (NCT02645981). The study is to explore the efficacy and safety of donafenib combined with TACE and anti-PD-1 antibody treatment in patients with uHCC. **Methods:** This single-center retrospective study enrolled unresectable HCC patients with ≥ 1 measurable lesion (mRECIST) who were treated with donafenib (200/100 mg po bid) + TACE + anti PD-1 antibody in Eastern Hepatobiliary Surgery Hospital. Best overall response (BOR), objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), changes from baseline in AFP/PIVKA levels and adverse events (AE) were collected. **Results:** 25 patients were enrolled (22 males, 24 ECOG of 1, aged 26-74 years, 23 HBV positive, 21 BCLC stage C, 18 as first-line and 7 as second-line) from June through December 2021. Baseline AFP level was ≥400 ng/mL among 11 (44.0%) patients. At data cut-off (March 1, 2022), the median follow-up time was 3 months (range 1-7). The BOR had 4 complete response (16.0%), 13 partial response (52.0%), 5 stable disease (20.0%) and 3 progressive disease (12.0%), resulting in ORR of 68.0% (95% CI, 46.5% - 85.1%) and DCR of 88.0% (95% CI, 68.8% - 97.5%). PFS rate at 6 months was 59.9% (95% CI 35.3% - 77.7%, mPFS was not reached). Patients treated in the first-line setting had better ORR (77.8%) and DCR (94.4%) than those treated in the second-line setting (42.9% and 71.4%, respectively). AFP and PIVKA decreased in 69% and 49.7% patients respectively. Nineteen (76.0%) patients reported AEs, including 7 (28.0%) with grade 3 AEs. No grade 4/5 AEs or serious adverse event was reported. The most frequent grade 3 AEs were rash (16%) and hand-foot skin reactions (8.0%). **Conclusions:** TACE combined with donafenib and anti-PD-1 antibody showed encouraging efficacy and good safety profile in patients with uHCC. **Keywords:** unresectable hepatocellular carcinoma; donafenib combined with TACE; efficacy; safety; anti-PD-1 antibody.

S13-6

Integrative multi-omics identifies immune subgroups of intrahepatic cholangiocarcinoma with distinct therapeutic vulnerabilities

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Aims: Tumor microenvironment plays a critical role in the selection of subsequent treatment strategies, especially immunotherapy. However, the microenvironment landscape of iCCA remains to be fully determined. We aimed to delineate the immunological features of iCCA and provide potential therapeutic targets. **Methods:** We performed genomic, transcriptomic, and proteomic characterization of 255 paired iCCA and adjacent liver tissues. We validated our findings through H&E staining (n = 177), multiplex immunostaining (n = 188), scRNA-seq (n = 10), in vitro functional studies and in vivo transposon-based mouse models. **Results:** Integrated multi-omics data identified three immune subgroups with distinct clinical, genetic, and molecular features, designated as IG1 (immune-suppressive, 25.1%), IG2 (immune-exclusion, 42.7%), and IG3 (immune-activated, 32.2%). IG1 was characterized by excessive infiltration of neutrophils and immature DCs. The hallmark of IG2 was the relatively higher tumor proliferative activity and tumor purity. IG3 exhibited an enrichment of adaptive immune cells, NK cells, and activated DCs. These immune subgroups were significantly associated with prognosis and validated in two independent cohorts. Tumors with KRAS mutations were enriched in IG1 and associated with myeloid inflammation-dominated immunosuppression. Although tumor mutation burden was relatively higher in IG2, loss of heterozygosity in human leukocyte antigen and defects in antigen presentation undermined the recognition of neoantigens, contributing to immune-exclusion behavior. Pathological analysis confirmed that tumor-infiltrating lymphocytes and tertiary lymphoid structures were both predominant in IG3. HBV-related samples tended to be underrepresented in IG1, and scRNA-seq implied that HBV

infection indeed alleviated myeloid inflammation and reinvigorated anti-tumor immunity. **Conclusions:** Our study elucidates that the immunogenomic traits of iCCA is intrinsically heterogeneous among patients, posing great challenge and opportunity for the application of personalized immunotherapy. **Keywords:** unresectable hepatocellular carcinoma; donafenib combined with TACE; efficacy; safety; anti-PD-1 antibody.

S13-7

Predicting the efficacy of lenvatinib plus anti-PD-1 antibodies in unresectable hepatocellular carcinoma (uHCC) using radiomics features of tumors extracted from baseline MRI

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Background: Combination therapy with lenvatinib plus anti-PD-1 antibodies is an effective treatment for advanced HCC. However, there are no established biomarkers to predict patient response to this combination therapy. **Methods:** This retrospective study enrolled consecutive patients with uHCC receiving first-line lenvatinib plus an anti-PD-1 antibody between Sep 2018 and Jun 2021 at Zhongshan Hospital and Liver Cancer Institute, with at least one radiological response evaluation. Intrahepatic tumor response was assessed every 2 months (± 2 weeks) by contrast-enhanced MRI using modified RECIST; patients with a best intrahepatic tumor response of complete or partial response were defined as radiological responders and those with stable or progressive disease were defined as radiological non-responders. Radiomic features of intrahepatic tumors were extracted from the enhanced arterial and delayed phase of baseline MRI images. A Least Absolute Shrinkage and Selection Operator (LASSO) model was employed for feature selection. A Neural Network was used to develop the prediction model. The optimal cutoff value was determined using a receiver operating characteristic (ROC) curve by maximizing the Youden index. **Results:** Of 117 eligible patients, 51.3% (n = 60) were classified as radiological responders and 48.7% (n = 57) as non-responders. All patients were randomly divided into a training (n = 94) and test (n = 23) set. A total of 2,236 radiomic features were extracted and normalized using z-score normalization. Features in the training set with intraclass correlation coefficients ≥ 0.80 were introduced into the LASSO model. Five features in the arterial phase and two in the delayed phase were identified as significant and used to build a neural network. The optimal cutoff value was 0.374. The area under the ROC curve, accuracy, specificity, sensitivity, positive predictive value and negative predictive value for predicting objective response were 0.918 ($P < 0.001$), 87.2%, 93.5%, 81.2%, 92.9% and 82.7% in the training set, respectively, and were 0.788 ($P = 0.009$), 73.9%, 100%, 50.0%, 100% and 64.7% in the test set, respectively. **Conclusions:** Radiomics features from baseline MRI may serve as predictors for objective response to lenvatinib plus anti-PD-1 antibodies in patients with uHCC before treatment initiation. **Keywords:** unresectable hepatocellular carcinoma; combination therapy; predictor; radiomics features; baseline MRI.

Conference contribution Abstracts

HCC-clinical

HC-01

Translational hepatectomy for hepatocellular carcinoma with inadequate future-liver -remnant after portal vein ligation in combination with apatinib plus camrelizumab: A single-arm prospective pilot study (PLACES) compared with ALPPS cohort

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Aims: Oncologic progress is one of the major morbidities that fails the traditional two-step hepatectomy (TACE, PVL, PVE, ALPPS) while waiting for compensatory growth of the residual liver. The combination of anti-PD1 and anti-VEGF has remarkable therapeutic effect on advanced HCC. We aimed to evaluate the efficay and safety of hepatic PVL combined with apatinib and camrelizumab in conversion of HCC with insufficient FLR to resectable HCC. **Methods:** We conducted an open, single-arm, prospective phase II clinical trial (PLACES study). Patients enrolled were first treated with endoscopic or open ligation of the portal vein on the diseased side of the liver, and then given apatinib 250 mg qd orally, combined with camrelizumab 200 mg ivgtt q2w, starting 2 weeks after surgery. Stage-II resection indication was that FLR/standard liver volume (SLV) increased above 40% for cirrhotic liver or 30% for normal liver, without contralateral and extrahepatic metastasis. Then the TKI+PD1 medication was stopped for 4 weeks until the offstage-II liver tumor resection. The TKI+PD1 medication continued for 1 year. The primary endpoints were translational resection rate, DFS, and the secondary endpoints were OS and incidence of AEs. The results of this study were also compared with propensity score matching (PSM) cases of ALPPS at the same center. **Results:** The median interval between the two operations was 111.5 days (75-217 days). One case of primary PVL had a grade 3 or higher adverse event, which was an elevation of aspartate transaminase (AST), the most common treatment-related adverse events for apatinib and camrelizumab included hypoalbuminemia (56.7%) and AST elevation (53.3%). Four patients with major adverse reactions (>grade 3) occurred after hepatectomy for stage II liver tumors, including thrombocytopenia, elevated AST, respiratory failure and pneumonia. During the maintenance period of combined treatment after hepatectomy, the most common adverse events were hypoalbuminemia (58.3%) and skin rash (50%). From 2012 to 2021,76 HCC patients received ALPPS, and the baseline data of both groups were matched 1:1 using PSM. The median follow-up time was 19.4 months (7.2-50.5 months). In comparison with ALPPS group, the OS ($P = 0.0017$) and DFS ($P = 0.0012$) were favorable for the PLACES group. **Conclusion:** PVL combined with apatinib and camrelizumab is an

effective and safe treatment option for HCC with insufficient residual liver volume, with higher minimally invasive rates and less postoperative morbidities than the ALPPS approach. **Keywords:** hepatocellular carcinoma; translational therapy; immunotherapy; targeted therapy.

1:1 PSM	PLACES group (n=25)	ALPPS group (n=25)	P value
Minimally invasive rate	88%	0%	P < 0.05
Operative time	213 ± 66 min	327 ± 74.7 min	P < 0.05
Blood loss	64.2 ± 99.1 ml	294 ± 189 ml	P < 0.05
postoperative complications	40%	100%	P < 0.05
Underwent secondary hepatectomy	16 (64%)	18 (72%)	
Second-stage operation interval	131 ± 43.3 days	25.1 ± 32.8 days	P < 0.05
Total length of hospitalization	23.9 ± 14.8 days	37.6 ± 10.4 days	P < 0.05
Cost of hospitalization	84977(35746-101228) Yuan	141590(117613-176968) Yuan	P < 0.05
FLR before the second stage of operation	579 ± 132 cm^3	754 ± 78.7 cm^3	P < 0.05
Kinetic growth rate before the second stage of operation	1.3 ± 0.8 cm^3	10.5 ± 7.9 cm^3	P < 0.05

HC-02

Early serum tumor marker responses predict the efficacy of lenvatinib plus an anti-PD-1 antibody in hepatocellular carcinoma

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Aims: Combination therapy with lenvatinib plus anti-PD-1 antibodies has shown high anti-tumor activity in hepatocellular carcinoma (HCC). However, predicting the efficacy of this combination therapy remains a challenge. This study aims to predict tumor response with the dynamic changes of serum biomarkers. **Methods:** Consecutive patients with unresectable HCC receiving lenvatinib and an anti-PD-1 antibody as first-line therapy at our hospital were included. Serum tumor markers, including alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II), were evaluated at baseline and 2-3 weeks after therapy. Associations between AFP/PIVKA-II response, radiological response and long-term survival were analyzed. **Results:** Ninety-eight patients were eligible. Baseline AFP or PIVKA-II was not associated with radiological response. Patients with a ≥50% AFP decrease had a higher rate of radiological response than those with an AFP increase or a <50% AFP decrease (75.9% vs 32.3%, P=0.001), and favorable progression-free survival (P=0.024). Patients with a ≥50% PIVKA-II decrease had a higher rate of radiological response than those with a PIVKA-II increase or <50% PIVKA-II decrease (75.0% vs 40.4%, P=0.005), and favorable overall survival (P=0.043). **Conclusion:** Early AFP or PIVKA-II decreases may predict objective response and long-term survival in patients with HCC receiving lenvatinib plus immunotherapy. **Keywords:** alpha-fetoprotein; anti–PD-1 antibody; hepatocellular carcinoma; lenvatinib; prediction; protein induced by vitamin K absence or antagonist-II.

HC-03

A novel indicator QD reveals the response evaluation regularity of liver cancer patients in the COVID-19 era

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Aims: The COVID-19 epidemic has disrupted the daily life of all people, especially cancer patients. In clinical practice, the evaluation cycle of patients is not regular, which impacts subsequent treatment and tumor monitoring to some extent. The objective of this study was to compare the characteristics and survival outcomes of patients with liver lesions based on the regularity of response evaluation (RE). **Methods:** Data on patients with tumor lesions in the liver between January 1, 2015, and December 31, 2021, were extract from the Clinical Research Intelligence System of Shanghai General Hospital. The quartile deviation (QD) of patient evaluation intervals was introduced as an indicator to determine the regularity of their voluntary RE. The characteristics, progression-free survival (PFS) and overall survival (OS) of patients from the start of treatment were evaluated and compared. **Results:** A total of 587 records from 102 patients were screened and analyzed, and two-thirds of the patients (60.784%) had more than 7 REs during the treatment period. The average QD value of all patients was 6.77, which was used to divide patients into a regular RE group and an irregular RE group. The regular group were younger (61.6 ± 1.218) and had shorter RE intervals (10.57 ± 0.4566) and better OS (mOS = 39 months). **Conclusion:** The QD of patient evaluation intervals is a reasonable indicator of RE regularity, and the regular administration of response evaluations in cancer patients is an important factor affecting survival outcomes. **Keywords:** response evaluation; liver cancer; regularity; quartile deviation; survival outcome.

HC-04

Perioperative and oncologic outcomes of laparoscopic versus open liver resection for combined hepatocellular-cholangiocarcinoma: A propensity score matching analysis

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Aims: Laparoscopic liver resection (LLR) has now been established as a safe and minimally invasive technique that is deemed feasible for treating hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC). However, the role of LLR in treating combined hepatocellular-cholangiocarcinoma (cHCC-CC) patients has been rarely reported. This study aimed to assess the efficacy of LLR when compared with open liver resection (OLR) procedure for patients with cHCC-CC. **Methods:** A total of 229 cHCC-CC patients who underwent hepatic resection (34 LLR and 195 OLR patients) from January 2014 to December 2018 in Zhongshan Hospital, Fudan University were enrolled and underwent a 1:2 propensity score matching (PSM) analysis between the LLR and OLR groups to compare perioperative and oncologic outcomes. Overall survival (OS) and recurrence-free survival (RFS) parameters were assessed by the log-rank test and the sensitivity analysis. **Results:** A total of 34 LLR and 68 OLR patients were included after PSM analysis. The LLR group displayed a shorter postoperative hospital stay (6.61 vs. 8.26 days; $p < 0.001$) when compared with the OLR group. No significant differences were observed in the postoperative complications' incidence or a negative surgical margin rate between the two groups ($p = 0.409$ and $p = 1.000$, respectively). The aspartate aminotransferase (AST), alanine aminotransferase (ALT), and inflammatory indicators in the LLR group were significantly lower than the OLR group on the first and third postoperative day. Additionally, OS and RFS were comparable in both the LLR and OLR groups ($p = 0.700$ and $p = 0.780$, respectively), and similar results were obtained by conducting a sensitivity analysis. **Conclusion:** LLR can impart less liver function damage, better inflammatory response attenuation contributing to a faster recovery, and parallel oncological outcomes when compared with OLR. Therefore, LLR can be recommended as a safe and effective therapeutic modality for treating selected cHCC-CC patients, especially for those with small tumors in favorable location. **Keywords:** combined hepatocellular-cholangiocarcinoma; laparoscopic; open liver resection; propensity score matching.

HC-05

Comparison of patient-reported outcomes between the combined lenvatinib and PD-1 inhibitors and the combined bevacizumab and PD-1/PD-L1 inhibitors in unresectable HCC

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Aims: The present study aimed to compare the effects of combined lenvatinib and PD-1 inhibitors versus combined bevacizumab and PD-1/PD-L1 inhibitors on patient-reported outcomes in unresectable HCC. **Methods:** Patient-reported outcomes was evaluated by the EORTC QLQ-C30 questionnaire monthly, which contained five functional domains (physical, emotional, role, cognitive, and social) and quality of life. The scores of each domain were transformed onto 0 to 100-point scale, with deterioration defined as a decrease from baseline of 10 points or more on the EORTC QLQ–C30 maintained for two consecutive assessments or a decrease of 10 points or more in one assessment followed by death from any cause within 1 month. **Results:** As of June 30, 2022, a total of 62 patients were enrolled, of which 34 received combined lenvatinib and PD-1 inhibitors, and 28 received combined bevacizumab and PD-1/PD-L1 inhibitors. The median time to deterioration of quality of life was 7.03 months in the lenvatinib + PD-1 inhibitor group, and that in the bevacizumab + PD-1/PD-L1 inhibitor group was not reached. There was no significant difference between the two groups (P=0.946). **Conclusion:** There was no significant difference in the impact of the two combination therapies on the quality of life of unresectable HCC patients, suggesting that the two regimens have comparable safety performance. **Keywords:** HCC; patient-reported outcome; target therapy; immunotherapy; combination therapy.

the median time to deterioration (month)	lenvatinib + PD-1 inhibitor	bevacizumab + PD-1/PD-L1 inhibitor	P value
physical function	4.87	13.07	P=0.258
role function	4.87	5.37	P=0.873
emotional function	8.50	12.97	P=0.096
cognitive function	2.87	3.40	P=0.840
social function	4.23	9.10	P=0.195

HC-06

Radiographic and α-fetoprotein response predict pathologic complete response to immunotherapy plus a TKI in HCC

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Aims: Pathologic complete response (pCR) following preoperative systemic therapy is associated with improved outcomes after subsequent liver transplant/resection in hepatocellular carcinoma (HCC). However, the relationship between radiographic and histopathological response remains unclear. **Methods:** We retrospectively examined patients with initially unresectable HCC who received tyrosine kinase inhibitor (TKI) plus anti–programmed death 1 (PD-1) therapy before undergoing liver resection between March 2019 and September 2021 across 6 hospitals in China. Radiographic response was evaluated using modified RECIST. A pCR was defined as no viable tumor cells in resected samples. **Results:** We included 35 eligible patients, of whom 15 (42.9%) achieved pCR after systemic therapy. After a median

follow-up of 13.2 months, tumors recurred in 8 non- pCR and 1 pCR patient. Before resection, there were 6 complete responses, 24 partial responses, 4 stable disease cases, and 1 progressive disease case, per mRECIST. Predicting pCR by radiographic response yielded an area under the receiver operating characteristic curve (AUC) of 0.728 (95% CI: 0.552-0.904, P=0.012), with an optimal cutoff value of 80% reduction in the enhanced area called major radiographic response), which had a 66.7% sensitivity, 85.0% specificity, and a 77.1% diagnostic accuracy. When radiographic response was combined with α-fetoprotein response, the AUC was 0.923 (95% CI: 0.818-1, P <0.001); the optimal cutoff value was 0.446, which had a 91.7% sensitivity, 84.6%, specificity, and an 88.0% diagnostic accuracy. **Conclusion:** In patients with unresectable HCC receiving combined TKI/anti–PD 1 therapy, major radiographic response alone or combined with α-fetoprotein response may predict pCR. **Keywords:** HCC; pathologic complete response; radiographic response; α-fetoprotein; combined TKI/anti–PD 1 therapy.

HC-07

To validate the efficacy of CD3+CD4+CD25hiCD127dimTreg in peripheral blood as a predictor for acute rejection in liver transplantation patients treated with immunocheckpoint inhibitors before surgery

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Aims: To investigate and verify the efficacy of noninvasive indicators for predicting acute rejection in liver transplantation patients treated with immunocheckpoint inhibitors before surgery. **Methods:** The proportion of CD3+CD4+CD25hiCD127dim regulatory T lymphocytes in peripheral blood were detected in patients receiving liver transplantation with different etiology (HCC patienst group, HCC patients treated with preoperative immune checkpoint inhibitors group, and liver cirrhosis or other liver diseases patients group) in during perioperative period and the incidence of acute rejection and clinical prediction were followed up. **Results:** We found that the increase of CD3+CD4+CD25hiCD127dim Treg in peripheral blood of patients who received immunocheckpoint inhibitors before surgery accompanied by high fever, leukopenia and other manifestations suggested that the higher risk of acute rejection in such patients and the variation trend of the number of Treg was inconsistent with that of other patients. Meanwilhe, since bariciximab may affect the expression level of CD25 on the surface of T lymphocytes, we try to establish a staining scheme for surface phenotypic markers of regulatory T lymphocytes that do not contain CD25 molecules to clarify the real changes of Treg which may provide a more comprehensive assessment of immune system changes in liver transplantation patients. **Conclusion:** The dynamic trend of CD3+CD4+CD25hiCD127dimTreg in peripheral blood can be used as a predictor of acute rejection in liver transplantation patients treated with immunocheckpoint inhibitors before surgery. **Keywords:** HCC; transplantation; CD3+CD4+CD25hiCD127dimTreg; immunocheckpoint inhibitor; acute rejection.

HC-08

Criteria for identifying potentially resectable patients with initially oncologically unresectable hepatocellular carcinoma before treatment with lenvatinib plus an anti-PD-1 antibody

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Aims: Conversion surgery has been reported to be feasible in patients with oncologically unresectable hepatocellular carcinoma (HCC). However, it is hard to identify the subset of patients who are more likely to undergo conversion surgery before initiating systemic therapy or combined with locoregional therapy. **Methods:** Criteria for identifying potentially resectable patients with initially oncologically unresectable HCC before treatment with lenvatinib plus an anti-PD-1 antibody were proposed based on real-word evidence in this study. An untouched cohort with consecutive patients who had advanced HCC and received combination therapy with lenvatinib plus an anti-PD-1 antibody between September 2018 and September 2021 were retrospectively enrolled to validate the proposed criteria using multivariate firth logistic regression. **Results:** The proposed criteria were as follows: (1) patients who have Eastern Cooperative Oncology Group performance status of 0-1; (2) patients who have Child-Pugh class A; (3) intrahepatic tumors are confined to one lobe (left, right, or middle lobe), or combined with a single tumor with diameter ≤5 cm or up to 3 tumors each with diameter ≤3 cm in the remaining lobe. R0 resection can be achieved by hemihepatectomy or combined with locoregional therapy in the remaining lobe during surgery; (4) portal vein tumor thrombus should not involve the contralateral liver lobe and not reach the superior mesenteric vein. Meanwhile, hepatic vein tumor thrombus involves no more than two major hepatic vein branches on the tumor side, and the tumor thrombus of inferior vena cava do not reach the atrium. The firth logistic regression confirmed the criteria as an independent predictor for undergoing conversion surgery. **Conclusion:** This study proposed and validated criteria for identifying the potentially resectable patient population who had initially oncologically unresectable HCC before initiating combination therapy with lenvatinib plus an anti-PD-1 antibody. The proposed criteria could be used as a reference for conversion research in advanced HCC. **Keywords:** anti–PD-1 antibody; combination drug therapy; criteria; hepatocellular carcinoma; Lenvatinib; potentially resectable.

HC-09

Clinical analysis of thirty-nine cases with peliosis hepatis

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Aims: Peliosis Hepatis (PH) is a rare benign lesion of the liver, which is characterized by multiple distributed, bloodfilled cystic areas of variable size in the liver parenchyma. The formation of PH is associated with malignancy, immunosuppression, drug administration and infection. Due to its nonspecific imaging findings, PH is frequently misdiagnosed as hepatocellular carcinoma,

metastatic lesions, or multiple abscesses. This research is intended to summarize the characteristics of PH diagnosis and treatment and improve understanding of PH. **Methods:** The past medical history, laboratory and imaging examination, and pathological features of 39 patients with PH were retrospectively analyzed. **Results:** From January 2008 to December 2017, there were 39 patients with PH confirmed by pathology in our department, accounting for 0.17% (39/23020) of the total number of liver operations during the same period. There were 21 males and 18 females, with a median age of 54 years. Seven (17.9%) PH patients had a history of extrahepatic malignant tumors (colorectal cancer or gastric cancer) and had received systemic chemotherapy before, but no recurrence and metastasis were identified during the operation. Three cases (7.7%) had synchronous liver metastases from colon cancer. PH co-existed with benign hepatic tumors (hepatic adenoma or hepatic hemangioma) in 9 cases (23.1%) and with hepatocellular carcinoma in 11 patients (28.2%). Nine (23.1%) PH patients had no other liver lesions and no history of malignant tumor. Thirty-three cases (84.6%) had solidarity PH, and 6 cases (15.4%) had multiple PH, with a median diameter of 2.0 cm (0.2-7 cm). All PH patients were undiagnosed preoperatively. **Conclusion:** Due to the rarity of PH, it is often misdiagnosed as other lesions before surgery. The possibility of PH should be considered in differential diagnosis. Clinicians and radiologists must be aware of the presence of this lesion to minimize misdiagnosis and overtreatment. **Keywords:** peliosis hepatis; liver lesions; differential diagnosis.

HC-10

Effectiveness and safety of lenvatinib plus anti-PD-1 antibodies in patients with hepatocellular carcinoma: a real-world cohort study

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Aims: Lenvatinib plus anti-programmed death-1 (anti-PD-1) antibody combinations have shown potent anti-tumor effect in phase I/II trials in advanced or unresectable hepatocellular carcinoma (HCC), but real-world data are limited. **Methods:** To investigate the effectiveness and safety of lenvatinib plus anti-PD-1 antibodies in a real-world cohort, we retrospectively evaluated 210 patients with unresectable or advanced HCC treated with these regimens between October 2018 and February 2022. **Results:** The objective response rate and disease control rate per RECIST v1.1 was 28.1% and 75.2%. Median overall survival (OS) and progression free survival (PFS) in the overall cohort were 17.2 and 8.4 months, respectively. Median OS and PFS of patients receiving first-line treatment reached 18.9 and 9.6 months. Median OS was significantly longer in patients with Child–Pugh class A vs B (18.8 vs 5.9 months, respectively), as was median PFS (9.1 vs 4.4 months). Patients with albumin–bilirubin (ALBI) grade 1 vs grade 2/3 also had significantly greater median OS (23.5 vs 13.4 months). Treatment-related adverse events (AEs) occurred in 79.5% of patients. Patients with ALBI grade 2/3 had a higher rate of grade 3/4 adverse events than patients with ALBI grade 1 (57.5% vs 38.5%). **Conclusion:** Lenvatinib combined with anti-PD-1 antibody therapy was effective in a large real-life cohort including patients with poor baseline liver function. Child–Pugh class and ALBI grade showed robust prognostic significance. Further study is needed to improve therapeutic efficacy and adverse event management in patients with Child–Pugh class B or ALBI grade 2/3. **Keywords:** ALBI grade; anti-PD-1 antibody; Child–Pugh class; hepatocellular carcinoma; lenvatinib

HC-11

Development of a pathological estimation score for predicting hepatocellular carcinoma recurrence after liver transplantation using artificial intelligence

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Aims: Hepatocellular carcinoma (HCC) recurrence occurs in 10-15% of patients after LT, with a high frequency of extrahepatic metastasis including bones and lungs. Traditional transplantation criteria and clinical biomarkers fail to precisely predict the post-LT recurrence. The study aimed to develop a pathological estimation score for predicting tumor recurrence after LT (PETRALT) using artificial intelligence (AI). **Methods:** Two datasets of 380 HCC patients underwent LT and 495 digital whole slide images (WSIs) were enrolled. Residual convolutional neural networks were used to identify six histological structures of HCC (tumor region, normal liver tissue, portal area, immune cells, fibrous tissue, and hemorrhage/necrosis area). Individual risk score of each tissue category was calculated by modified Deepsurv network using recurrence status and time to recurrence as labels. PETRALT was constructed according to the multivariate analysis. The model discrimination was estimated by receiver operating characteristic (ROC) curve with area under curve (AUC) value and concordance index (C-index). The Kaplan-Meier curve was used to compare survival in different risk subgroups. The cellular exploration of prognostic immune biomarkers was performed by three panels of 7-color immunofluorescence. **Results:** The overall classification accuracy of HCC tissue was 97%. In the structural level, immune cell was the only significant tissue category for predicting post-LT recurrence (HR= 1.907, 95% CI: 1.490-2.440). The C-indexes of PETRALT achieved 0.808 and 0.768 in the training and validation cohorts, respectively. PETRALT outperformed transplantation criteria, tumor stages and clinical models in post-LT recurrence prediction (AUC= 0.825). Multivariate cox regression analysis for recurrence-free survival (RFS) showed that PETRALT (HR= 5.629, 95% CI: 3.527-8.983) was an independent risk factor. Patients in the high-risk subgroup had a shorter RFS, elevated serum alpha fetoprotein level and a higher proportion of micro-vascular invasion. In the cellular level, a higher infiltration of intratumoral NK cells was negatively correlated with recurrence risk (p= 0.01). An immunosuppressive tumor microenvironment was observed in the early-recurrence cases. **Conclusion:** The study established and validated a pathological risk score using AI. Immune cell was the most significant histological structure related to HCC recurrence. PETRALT performed well in post-LT recurrence prediction and indication of clinicopathological features. **Keywords:** hepatocellular carcinoma; liver transplantation; recurrence; artificial intelligence; immune cells.

HC-12

Postoperative adjuvant trans-arterial chemoembolization for patients with hepatocellular carcinoma and bile duct tumor thrombus

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Aims: This study aims to explore the efficacy of postoperative adjuvant transarterial chemoembolization (PA-TACE) for patients with hepatocellular carcinoma (HCC) and bile duct tumor thrombus (BDTT) and to determine the prognosis efficacy. **Methods:** From January 2005 to June 2015, HCC patients with histologically confirmed BDTT and well-tolerated liver function who underwent PA-TACE after liver resection (LR) or LR alone were studied retrospectively. In the PA-TACE group, PA-TACE was given 4 weeks after LR. Survival rates of the patients were analyzed by the Kaplan-Meier method. Cox's proportional hazard model was used to investigate the risk factors for disease-free survival (DFS) and overall survival (OS). **Results:** Of the 125 HCC patients with BDTT included in the analysis, 58 (46.4%) entered into the LR group and 67 (53.6%) entered into the PA-TACE group. The baseline characteristics of the two groups were similar except for serum total bilirubin level (p = 0.013). The 1-, 2-, 3-, and 5-year DFS rates were respectively 68.9, 56.8, 43.7, and 34.7 % for the PA-TACE group and 49.3, 33.1, 27.1, and 22.3 % for the LR group (p = 0.017). The 1-, 2-, 3- and 5-years OS rates were 72.6%, 59.2%, 47.0% and 38.3% in the LR group and 87.0%, 74.2%, 64.2% and 56% in the PA-TACE group, respectively (p = 0.005). Multivariate Cox proportional hazards regression analysis showed PA-TACE to be an independent risk factor of postoperative DFS and OS. **Conclusion:** Postoperative adjuvant TACE should be regarded as a common strategy for HCC patients with BDTT. **Keywords:** bile duct tumor thrombus; postoperative adjuvant transarterial chemoembolization; hepatocellular carcinoma; prognosis; survival.

HC-13

Lusutrombopag for the treatment of thrombocytopenia in Chinese patients with chronic liver disease undergoing elective invasive procedures: a randomized, multicenter, double-blind phase 3 study

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Aims: Lusutrombopag has been approved for treating thrombocytopenia associated with chronic liver disease (CLD) in patients who are scheduled to undergo invasive procedures in U.S., Europe, and Japan. We aimed to evaluate the efficacy and safety of lusutrombopag in Chinese patients with CLD-associated thrombocytopenia undergoing elective invasive procedures. **Methods:** This multicenter, randomized, double-blind, placebo-controlled phase 3 trial (CTR20192384) was conducted in 9 centers in China. Patients over 18 who had severe thrombocytopenia (platelet [PLT] counts $<50\times10^9$/L) associated with CLD, and were scheduled to undergo invasive procedures (excluding thoracotomy, laparotomy, open-heart surgery, craniotomy, organ or partial organ resection) were enrolled. Eligible patients were 2:1 randomly assigned to receive once-daily oral 3 mg of lusutrombopag or placebo from day 1 to day 7. The scheduled invasive procedures were performed between day 9 and day 15. Preoperative platelet transfusion was allowed in patients with the PLT $<50\times10^9$/L. The primary endpoint was the proportion of responders (defined as the patients with PLT $\geq50\times10^9$/L and with an increase of PLT $\geq20\times10^9$/L from baseline with no rescue therapy for bleeding) on day 8. The key secondary endpoint was the proportion of patients with PLT $\geq50\times10^9$/L on or after day 8 and within 2 days (the alternative criterion that does not require platelet transfusion) prior to the invasive procedure. Adverse events (AEs) were also recorded. **Results:** From July 2020 to June 2021, 66 patients were enrolled. Lusutrombopag had potent effect of platelet elevation and was well tolerated. All of the AEs were mild or moderate in severity. Only one patient in the lusutrombopag group reported thrombosis-related AEs (brachiocephalic vein thrombosis), which was mild and determined not related to the treatment. Three bleeding events in three patients occurred in the lusutrombopag group, and four bleeding events in three patients reported in the placebo group (6.8% vs. 13.6%). **Conclusion:** Lusutrombopag was effective in increasing PLT and

	lusutrombopag group (n=44)	placebo group (n=22)	P value
The proportion of responders on day 8	43.2% (19/44)	4.5% (1/22)	P=0.0011
The proportion of patients with PLT $\geq50\times10^9$/L on or after day 8 and within 2 days prior to the invasive procedure.	72.7% (32/44)	18.2% (4/22)	P < 0.0001
the responder rates at any time	81.8%	54.5%	
the proportion of patients who require rescue therapy for bleeding at any time	0	0	
The median maximum PLT	80.5×10^9/L	60.0×10^9/L	
The median maximum increase of PLT from baseline	42.0×10^9/L	24.0×10^9/L	
The median time to reach the maximum PLT	14.5 days	27.0 days	
Incidence of drug-related AEs	11.4%	18.2%	

avoiding preoperative platelet transfusion in Chinese patients with CLD-associated thrombocytopenia scheduled to undergo invasive procedures, with comparable safety profiles to placebo. **Keywords:** chronic liver disease; thrombocytopenia, thrombopoietin receptor agonist; lusutrombopag.

HC-14

Survival prediction for unresectable hepatocellular carcinoma after hepatic arterial infusion chemotherapy using convolutional neural network with radiologic and clinical factors

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Aims: To evaluate the feasibility of convolutional neural network (CNN) with preoperative MRI and clinical factors to predict overall survival (OS) and progression-free survival (PFS) of unresectable hepatocellular carcinoma (HCC) patients treated with hepatic arterial infusion chemotherapy (HAIC). **Methods:** 191 patients with unresectable HCC after HAIC from Zhongshan Hospital between May 2019 and March 2022 were retrospectively recruited in this study. Radiomics scores were calculated based on enhanced-T1-weighted, enhanced-T2-weighted, arterial phase and delayed phase images. The CNN was constructed on 70% of the data set and tested on the remaining 30%, which was composed of two convolutional, two maximum pooling, one fully connected layer and two linear mapping layers. Clinical factors related to OS and PFS were identified by Cox regression analysis. Radiomics scores and clinical factors were reflected to a model by AlexNet. Accuracy, loss, time-dependent receiver operating characteristic curve and prediction error were calculated for the models. **Results:** The OS model included radiomics score with No. of HAIC cycles, tumor thrombus, PIVKA-II, neutrophil-lymphocyte ratio (NLR), aspartate aminotransferase (AST), gamma-glutamyltranspeptidase (γ-GT) and C-reactive protein. And the PFS model included radiomics score with No. of HAIC cycles, tumor thrombus, NLR and γ-GT. The OS model achieved an accuracy of 0.836 and the PFS model achieved an accuracy of 0.700. **Conclusion:** The proposed models integrated radiomics information and clinical factors helped predict OS and PFS of unresectable HCC patients after HAIC. **Keywords:** unresectable hepatocellular carcinoma; hepatic arterial infusion chemotherapy; convolutional neural network; survival prediction.

HC-15

Liver function in patients with advanced hepatocellular carcinoma following combination therapy using lenvatinib and an anti-PD-1 antibody

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Aims: Combination therapy with lenvatinib and an anti-programmed death 1 (PD-1) antibody in patients with advanced hepatocellular carcinoma (HCC) shows efficacy for tumor response and survival. We aim to assess the effect of this combination therapy on liver function. **Methods:** This retrospective study included patients with advanced HCC who received 3 months of combination treatment at our hospital from August 2018 to October 2020 and had at least one efficacy and safety assessment. **Results:** A total of 112 patients were screened and 79 were eligible for this study. Average ALBI scores decreased from −2.584 ± 0.391 at baseline to −2.707 ± 0.448 following 3-months of treatment (P < 0.01). ALBI scores of patients with baseline ALBI grade 1 did not change over the study period (baseline: −2.89 ± 0.23; 3 months: −2.84 ± 0.41; P = 0.844), while those with ALBI grade 2 decreased from −2.29 ± 0.26 to −2.58 ± 0.45 at month 3 (P < 0.001). Furthermore, patients with baseline ALBI grade 2 who achieved partial response or stable disease had significant reductions in ALBI score during treatment, but not those with progressive disease. Positive HBV-DNA replication at baseline in patients receiving antiviral therapy exhibited similar ALBI scores as those with negative HBV-DNA replication. **Conclusion:** Combined therapy did not cause significant liver function impairment during early-stage of treatment. A decreased tumor burden was associated with recovery of liver function patients with baseline ALBI grade 2. Positive HBV-DNA replication did not affect liver function in patients receiving antiviral therapy. **Keywords:** hepatocellular carcinoma; albumin-bilirubin grade; lenvatinib; PD-1; Liver reserve function.

HC-16

Dynamic monocyte index predicts prognosis of patients after curative resection for hepatocellular carcinoma

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Aims: To develop a novel dynamic monocyte index (DMI) and to explore its prognostic value for overall survival (OS) and time to recurrence (TTR) in hepatocellular carcinoma (HCC) patients. **Methods:** The DMI was developed based on a training cohort (n = 755) and verified in 3 validation cohorts (n = 123, 100, 161). The expression of CD3, CD4, CD8, Chemokine (C-C motif) ligand 2 (CCL2), CD68, FOXP3 (T-regulatory cells, Tregs), programmed death protein 1 (PD-1), and programmed death protein ligand 1 (PD-L1) were assessed by immunohistochemistry (IHC) in tissue microarrays, and circulating tumor cells (CTCs) in peripheral blood of 123 patients were detected before surgery. **Results:** Patients in the training cohort were stratified into high DMI group (DMI > 0) and low DMI group (DMI ≤ 0) with an optimal DMI cutoff point of 0.0 for TTR. Cox multivariate analyses confirmed DMI as an independent predictor for OS and TTR in the training cohort and 3 validation cohorts, respectively. DMI was negatively correlated with CCL2 IHC score in peritumor (P <0.001), density of macrophages (CD68+ lymphocytes, MΦ) in peritumor (P <0.001), PD-1+ lymphocytes in peritumor (P = 0.026), and PD-L1 in peritumor (P = 0.039). In particular, compared with negative CTCs patients, DMI showed better prognostic value for TTR (P = 0.003 vs P = 0.693) in positive CTCs (≥1) patients. **Conclusion:** The DMI is a promising prognosis biomarker for HCC patients underwent resection and is a novel indicator for HCC therapeutic approaches choosing. The dismal prognosis in low DMI group

may be associated with higher monocytes recruitment and immunosuppressive microenvironment, which facilitated CTCs recolonization in peritumor after surgery. **Keywords:** dynamic monocyte index; prognosis; hepatocellular carcinoma.

HC-17

GP73 activates JAK/STAT3 signaling pathway to promote angiogenesis in HCC

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Aims: Studies have confirmed that GP73 is closely associated with the HCC cells proliferation, migration, invasion, EMT, exosomes, ERS and drug resistance, but there is no report on the relationship between GP73 and angiogenesis. We had found the vascular invasion of HCC patients with GP73 overexpression was more significant. Therefore, we further explored the role of GP73 in the angiogenesis of hepatoma cells and its molecular mechanism. **Methods:** We established several stable transfected cell lines to control GP73 expression. Then we detected the expression of key genes of JAK/STAT3 signaling pathway including STAT3, MCL-1 and BCL-2 by RT-qPCR and WB, and detected the transfection efficiency, the protein level of STAT3 mutation sites and the effect of GP73 knockdown and overexpression on the phosphorylation level of STAT3 by WB. CCK8, Transwell and Matrigel tubule formation assays were used to detect the effects of GP73, STAT3, STAT3 mutation sites and C188-9 inhibitors on the proliferation, invasion, migration and angiogenesis of HUVECs. Gene chip was used to detect the effect of GP73 on angiogenesis-related indexes. The relationship between GP73 and STAT3 was detected by Co-IP, molecular docking, immunofluorescence and luciferase experiments. **Results:** The ability of HUVECs proliferation, invasion and angiogenesis were suppressed and increased respectively in the 97H-GP73-KD and Hep3B-GP73-OE cells. LIF, STAT3, MCL-1, and BCL-2 were significantly decreased, while IL15RA, OSMR, JAK2 and JAK3 were significantly increased in 97H-GP73-KD cells, the change of the mRNA and protein expression level were consistent. There was no significant difference with the control group. However, overexpression of GP73 achieved the opposite effect. In the Hep3B-GP73-OE cell line, knockdown of STAT3 expression and the use of C188-8 inhibitor, the HUVECs proliferation, migration and tubulogenesis were attenuated. Overexpression of GP73 promoted the up-regulation of angiogenic factors CXCL5, MCP-1 and TPO. The protein expression of p-STAT3 (Ser727) was significantly reduced after GP73 knockdown, and increased after GP73 overexpression. Compare with the 97H-GP73-KD, the 97H-GP73-KD-STAT3-OE, 97H-GP73-KD-STAT3-Tyr705 and 97H-GP73-KD-STAT3-Ser727 groups HUVECs proliferation, migration, and angiogenesis abilities were significantly increased. The results of Co-IP, molecular docking, immunofluorescence staining and luciferase experiments showed that GP73 interacted with STAT3 and co-localized. **Conclusion:** This study is the first to demonstrate that GP73 is a potential anti-angiogenesis therapeutic target in HCC that activates the JAK/STAT3 signaling pathway by targeting and promoting the phosphorylation expression of STAT3 to promote HCC angiogenesis. **Keywords:** HCC; GP73; angiogenesis; STAT3 phosphorylation; JAK/STAT3.

HC-18

Genomic and epigenomic abnormalities in plasma cfDNA as liquid biopsy biomarkers for the diagnosis of hepatocellular carcinoma

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Aims: Previous studies have shown that surveillance of people at high risk of HCC by combining alpha-fetoprotein (AFP) and ultrasonography can achieve early detection and diagnosis of HCC, reduce the mortality rate. However, the sensitivity of ultrasonography for early HCC is relatively limited, and the sensitivity and specificity of AFP for HCC are also suboptimal, limiting its clinical applications. Meanwhile, blood and other body fluids contain circulating free DNA (cfDNA) shed by tumors, which carries genomic and epigenomic alterations that accumulate during tumorigenesis, providing new opportunities for non-invasive detection of HCC. We aimed to investigate the genomic and epigenomic abnormalities in plasma cfDNA as biomarkers for the diagnosis of HCC. **Methods:** In this study, we performed comprehensive cfDNA genomic profiling and methylome profiling by targeted ultra-deep sequencing and targeted bisulfite sequencing respectively, in Chinese HCC patients and the control group including both healthy individuals and individuals at high risk for HCC (individuals with hepatitis B infection or cirrhosis). **Results:** The model based on genomic mutations carried by cfDNA achieved a 63.3% sensitivity and a 94.9% specificity in differentiating HCC (n=120) from healthy subjects (n=117) in the testing set, while the model based on methylated DNA markers (MDMs) achieved a 90.0% sensitivity and a 92.5% specificity in differentiating HCC (n=120) from healthy subjects (n=120) in the testing set. The diagnostic performance of MDMs was superior compared to genomic variants. Based on the MDMs identified and validated, we developed a simple, customized, and targeted bisulfite sequencing method named MBA-seq (Multiplex PCR-based Bisulfite Amplicon sequencing) that encompassed 25 HCC-specific MDMs. The MBA-seq-based diagnostic model achieved a sensitivity of 86.7% and a specificity of 90.1% in distinguishing HCC (n=120) from non-HCC controls (110 individuals with hepatitis B infection or cirrhosis, 73 individuals with benign liver tumor, and 120 healthy individuals) in the testing set. Furthermore, we developed a methylation-specific qPCR diagnostic assay based on a combination of 2 HCC-specific MDMs, which achieved a 78.4% sensitivity and a 93.0% specificity in differentiating HCC (n=134) from non-HCC controls (110 individuals with hepatitis B infection or cirrhosis, 122 individuals with benign liver tumor, and 123 healthy individuals), and a sensitivity of 69.9% for early-stage HCC (CNLC stage I, n=93) in the testing set. Both assays achieved significantly better performance than the serum protein marker AFP. **Conclusion:** Our results suggested that aberrant methylation patterns of cancer-derived cfDNA provide high-performance biomarkers for the diagnosis of HCC in healthy and high-risk populations. **Keywords:** circulating free DNA; hepatocellular carcinoma; methylation; diagnosis.

HC-19

Efficacy and safety of donafenib combined with anti-PD-1 antibody in the treatment of unresectable hepatocellular carcinoma: a retrospective real-world study

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Aims: The study is to explore the efficacy and safety of donafenib combined with anti-PD-1 antibody in patients with uHCC. **Methods:** This is a single-center retrospective study. The uHCC patients with≥ 1 measurable lesion (RECIST1.1) who were treated with donafenib (200 mg po bid) + anti PD-1 antibody in Southwest Hospital of AMU were enrolled. Data regarding objective response rate (ORR), disease control rate (DCR), and adverse events (AEs) were collected. **Results:** From 17-Sep-2022 to 21-Apr-2022, 28 patients were enrolled (25 males, 3 females; aged 24-72 years, median age 53). All the 28 patients were BCLC stage C and were treated with donofenib combined with anti-PD-1 antibody as first-line. Baseline AFP level was ≥400 ng/mL among 7 (25.0%) patients. The best overall response (BOR) included 2 complete response (7.1%), 4 partial response (14.2%), 19 stable disease (67.8%) and 3 progressive disease (10.7%), resulting in an ORR of 21.4% (95% CI 10.2%-39.5%) and DCR of 89.2 (95% CI, 72.7% - 96.2%). 2 (7.1%) patients reported grade 2 rash, no grade 4/5 AEs or serious adverse event were reported. **Conclusion:** Donafenib combined with anti-PD-1 antibody showed encouraging efficacy and good safety profile in patients with uHCC. **Keywords:** donafenib; anti-PD-1 antibody; uHCC; retrospective real-world study.

HC-20

Radiomics analysis of contrast-enhanced MRI predicting therapeutic response to lenvatinib-based systemic therapy in hepatocellular carcinoma

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Aims: To develop and verify a radiomics model based on pre-treatment contrast-enhanced magnetic resonance imaging (CE-MRI) for prediction of therapeutic response to lenvatinib-based systemic therapy in patients with hepatocellular carcinoma (HCC). **Methods:** 132 HCC patients underwent CE-MRI before lenvatinib-based systemic therapy between 2018.09 and 2020.12 were retrospectively enrolled and randomly divided into a training cohort (n = 107) and a validation cohort (n = 25). Best radiological response was evaluated according to the RECIST 1.1. There were 54 and 78 patients achieved OR and non-response, respectively. The region of interests (ROIs) were manually segmented along the outline of the tumor on each axial slice of T2-weighted imaging and enhanced arterial phase, portal venous phase and delayed phase T1-weighted imaging sequences. A total of 1142 radiomics features were extracted from each sequence, and maximal relevance and minimal redundancy (mRMR) and least absolute shrinkage and selection operator (LASSO) methods were applied to dimensionality reduction and feature selection. The radiomics models were developed based on selected features using the random forest method and their performance was evaluated by receiver operating characteristic (ROC) curve, calibration curve, and decision curve analysis. Univariate and multivariate logistic regression analyses were conducted to identify the independent risk factors for non-response to lenvatinib-based systemic therapy. The radiomics model which had the highest area under curve (AUC) was selected, and subgroup analysis and Kaplan-Meier survival analysis were performed to explore its applicability for various treatment regimens and prognostic value. **Results:** No significant differences were observed in the demographic and clinicopathological characteristics between the training cohort and validation cohort. After feature extraction and selection, 14 radiomics features were ultimately identified for radiomics model construction. The calibration curve confirmed that the T2 radiomics model had satisfactory consistency between the actual and predicted probability, and the decision curve analysis revealed its good clinical utility. None of the characteristics were independent predictors for therapeutic response except for the predicted results of the T2 radiomics model (P <0.001). Additionally, HCC patients who were predicted non-response by the T2 radiomics model had poorer overall survival (P = 0.017) and progression-free survival (P = 0.002). **Conclusion:** We developed a novel radiomics model based on T2-weighted imaging sequence, which was capable of predicting therapeutic efficacy and prognosis for patients with HCC undergoing lenvatinib-based systemic therapy. **Keywords:** hepatocellular carcinoma; radiomics; magnetic resonance imaging; systemic therapy; therapeutic response.

T2 radiomics model		AUC	95% CI
Training cohort		0.903	0.893 – 0.922
Validation cohort		0.885	0.863 – 0.909
Subgroup	Lenvatinib +TACE	0.972	0.971 – 0.982
	Lenvatinib +ICI	0.891	0.861 – 0.900
	Lenvatinib +ICI +TACE	0.894	0.887 – 0.922

HC-21

A summarized method of identifying cancer stem cell associated cancer initiation genes through hepatocellular carcinoma single cell landscape

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Aims: Hepatocellular carcinoma (HCC) has the second seriousness of lethal burden among malignancy worldwide. Continuous exploration on gene level contributes tremendously to improving prognosis consequence of HCC patients. This study

focused on the HCC single-cell sequencing data to discover significant variables closely related to cancer stem cells (CSCs). Cancer stem cell-related genes provide therapeutic strategy guidance on tumorigenesis orientation. **Methods:** The HCC single cell expression profile data was downloaded from Gene Expression Omnibus (GEO) database. The GSE166635 dataset using chromium 10X barcodes and containing two human HCC samples. To comprehensively indicate the tumor biological characteristics complexity and the internal theorem of malignant process, the single-cell RNA sequencing (scRNA-seq) was adopted. The gene-cell matrix identified distinct groups of cells using Seurat object construction. The cell clustering outcomes were calculated by PCA and t-SNE dimensionality reduction analysis. The Monocle2 was used to perform malignant cells pseudotime trajectory analysis. GSVA functional enrichment analysis of the differential expression features from TCGA-LIHC dataset confirmed the discovery. **Results:** After quality control and standard filtering, a total of 11577 cells were selected. The 6 clusters were identified through PCA and t-SNE dimensionality reduction analysis. Each cluster was defined according to cell marker genes. The representative gene of cancer stem cell and cellular-1 tumor cell are APOA2 and HMGB2. The study emphases on one cancer stem cell cluster and four cellular clusters to perform subsequent research. Pseudotime trajectory analysis exhibited three states of cell experience, including one initial branch and two developmental branches. Both cellular-1 cells and cancer stem cells were distributed within the initial branch. The gene set variation analysis (GSVA) analysis showed a similar function for the two clusters. **Conclusion:** This study defined a type of hepatocellular carcinoma and expounded on the connection between cellular type 1 cell and cancer stem cell. APOA2 and HMGB2 were identified as cancer initiation genes. Malignant initiation characteristics of cancer stem cells could be widely used for HCC treatments. The method we summarized had practical significance for cancer stem cell mediated immunotherapy. **Keywords:** hepatocellular carcinoma; cancer stem cell; initiation genes; single cell sequencing; pseudotime trajectory analysis.

HC-22

Impact of inflammation and fibrosis on post-hepatectomy prognosis of patients with hepatitis B virus-associated hepatocellular carcinoma without cirrhosis: A retrospective cohort study

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Aims: Inflammation and fibrosis are pathological manifestations of chronic liver disease and are strongly associated with the occurrence of hepatocellular carcinoma (HCC). Here, we investigated whether the degree of inflammation and fibrosis in paracarcinoma tissues can predict prognosis of the patients with hepatitis B virus (HBV)-associated HCC after hepatectomy. **Methods:** Clinicopathological data were retrospectively analyzed for 293 patients with HBV-associated HCC who were treated by curative resection from 2012 to 2017 at our institution. Patients were classified into two groups depending on Knodell score (≤7 or >7). Overall survival and recurrence-free survival rates were compared between the groups using Kaplan–Meier curves. Univariate and multivariate survival analyses were applied to identify the clinicopathological factors associated with HCC prognosis. Certain analyses were repeated on subsets of both groups matched 1:1 based on propensity scoring. **Results:** One third (33%) of our patients had established severe liver inflammation and fibrosis (Knodell score > 7). Rates of overall and recurrence-free survival at one, three, and five years were lower in patients with Knodell score > 7 than in those with Knodell score ≤ 7. Multivariate Cox regression analysis showed that Knodell score > 7 was an independent risk factor for overall survival and shorter recurrence-free survival. Moreover, Barcelona Clinic Liver Cancer C stage was an independent predictor of recurrence-free survival, while poor differentiated histology was independently associated with poor overall survival. **Conclusion:** The degree of inflammation and fibrosis measured by the Knodell score is a prognostic factor for patients with HBV-associated HCC. **Keywords:** inflammation; fibrosis; Knodell score; hepatocellular carcinoma; hepatitis B virus; prognosis.

HC-23

PD-1/PD-L1 blockade treatment in patients with hepatocellular carcinoma prior to liver transplantation: A high-volume single center retrospective study

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Aims: Due to reports of severe rejection, ICIs have not been thoroughly encouraged in patients with HCC before liver transplantation (LT). The clinical outcomes of pre-transplant ICIs, however, are still unknow. We aimed to investigate the safety and efficacy of PD-1/PD-L1 blockade in HCC patients before LT. **Methods:** A total of 16 HCC patients received primary LT from donation after citizens' death between January 2018 and December 2021 in our center were analyzed. Before LT, they all underwent PD-1/PD-L1 blockade. We excluded pediatric LT, ABO-incompatible LT and combined- or multi-organ transplantation. In our center, we routinely used a small dose of basiliximab and methylprednisolone during the peri-operative period. After LT, our immunosuppression regimen included combination or separate use of mycophenolate mofetil, corticosteroids, tacrolimus and sirolimus, which depended on the patient's condition. Commonly, post-transplant rejection was defined as biopsy-proven acute rejection (BPAR). In our study, significantly elevated transaminases or total bilirubin (TB) in post-transplant patients during the recovery stage of liver function, after exclusion of drug-induced graft injury, hepatic artery or portal vein embolism, was also considered as rejection. **Results:** All patients were male. The washout period between the last use of ICI and LT ranged from 8 days to 435 days. Allograft rejection occurred in 25.0% (4/16) of patients on postoperative day 3 (POD3) to POD167, among which 2 (12.5%) were BPAR. Of these 4 patients, 1 (25%) suffered from severe rejection on POD167, whose rejection activity index (RAI) was 7-8. Unfortunately, his transaminase and TB remained high

though tacrolimus, mycophenolate mofetil and corticosteroids were used to against rejection, and finally he died of BPAR on POD435. Another 3 patients who sustained mild post-transplant rejection all achieved well-controlled and discharged successfully. **Conclusion:** The current study indicated that the use of PD-1/PD-L1 blockade in patients before LT exhibited considerable efficacy in anti-tumor, as well as acceptable post-transplant rejection morbidity in recipients under close clinical supervising. Further multicenter and prospective research is needed in the future to explore the safety and efficacy of the use of ICIs for patients before LT. **Keywords:** hepatocellular carcinoma; liver transplantation; PD-1/PD-L1 blockade; rejection.

Characteristic Patients	
Total, n	16
Male, n	16
Age, mean (years)	56
Hepatitis B-related HCC, n (%)	15 (93.8%)
Received camrelizumab, n (%)	11 (68.8%)
Received tislelizumab, n (%)	2 (12.5%)
Received atezolizumab, n (%)	1 (6.25%)
Received toripalimab, n (%)	1 (6.25%)
Received cindilimab, n (%)	1 (6.25%)
Cycle of ICI treatment, median ± (SD)	3 (1 – 30)
Response rate (mRECIST)	
PR	4 (25.0%)
SD	6 (37.5%)
PD	6 (37.5%)
DCR	10 (62.5%)

HC-24

The efficacy and safety of donafenib-based treatments for patients with hepatocellular carcinoma: A retrospective single-centre real-world study

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Aims: Donafenib is a new multikinase inhibitor approved for advanced hepatocellular carcinoma (HCC) in patients without prior systemic therapy. We conducted a retrospective real-world study to evaluate the therapeutic efficacy and safety of donafenib for HCC patients in first-line and adjuvant settings. **Methods:** HCC patients with Child-Pugh class A or B who received donafenib treatment were enrolled between June 2021 to May 2022 in Zhongshan Hospital. Therapeutic efficacy was determined using the RECIST 1.1 and grades of adverse events (AEs) complied with the CTCAE 4.0. The patients with complete baseline information and at least one follow-up evaluation were finally included in this analysis. **Results:** A total of 133 patients were eligible for further analysis. In the first-line setting, the patients in CP-B showed a similar mDOT and discontinuation rate compared to the patients with CP-A. The most common ADRs (incidence ≥ 10%) in the first-line setting were hand-foot skin reaction (HFSR; 32/70, 45.7%), rash (10/70, 14.3%), diarrhea (9/70, 12.9%) and hypertension (8/70, 11.4%), while HFSR (20/63, 31.7%), rash (10/63, 15.9%) and diarrhea (7/63, 11.1%) in the adjuvant setting. The incidence of ADRs in 11 patients who had liver transplantation was 54.5% (grade 3: 9.09%), which was comparable to 53.8% (grade 3: 5.77%) in other patients in the adjuvant setting. The incidence of ADRs in patients with CP-B (69.2%) was the same as those with CP-A (69.2%), while there were more grade 3 ADRs in the former (15.4% vs 5.8%). There were more ADRs in 81 patients who received combination therapy (75.3%) than that in 52 patients who received donafenib alone (59.6%), while the incidence of grade 3 ADRs was similar (4.9% vs 9.6%). No grade 4 or 5 was reported in both settings. **Conclusion:** Donafenib alone or combined with TACE, ICI or both showed good tolerability and effectiveness for HCC patients with CP-A or B liver function in real-world practice, either as first-line treatment or in an adjuvant setting. **Keywords:** hepatocellular carcinoma; donafenib; real-world; first-line; adjuvant therapy.

Characteristic		**N (%)**
Total		133
Received donafenib as first-line treatment		**70**
Child-Pugh	Child-Pugh class A	59 (84.3%)
	Child-Pugh class B	11 (15.7%)
Viral infected	HBV infected	45 (64.3%)
China Liver Cancer Stage	China Liver Cancer stage I	15
	China Liver Cancer stage II	17

	China Liver Cancer stage IIIa	18
	China Liver Cancer stage IIIb	20
Treatment strategies	Received combination treatment	58 (82.9%)
	Donafenib + TACE	27 (38.6%)
	Donafenib + ICI+ TACE	23 (32.9%)
	Donafenib + ICI	7 (10.0%)
	Donafenib + ICI + HAIC	1 (1.4%)
Status of patients	Continued treatment	55 (78.6%)
Median duration of treatment (mDOT)	Continued treatment	5.0 months (IQR 2.0–6.5)
	Discontinued treatment	3.0 months (IQR, 1.0–5.5)
Disease control rate		88.6%
	Child-Pugh class A	90.9%
	Child-Pugh class B	88.1%
Median progression-free survival		Not reached
Adverse drug reactions (ADRs)	Any grade	58 (82.9%)
	Grade 3	5 (7.1%)
Received donafenib as adjuvant treatment after curative operation		**63**
Previous treatment strategies	Undergone curative resection	49 (77.8%)
	Received transplantation	11 (17.5%)
	Received tumor ablation	2 (3.2%)
Viral infected	HBV infection	41 (65.1%)
China Liver Cancer Stage	China Liver Cancer stage I	41
	China Liver Cancer stage II	11
	China Liver Cancer stage IIIa	11
Microvascular invasion (CNLC I-II)		18 (34.6%)
Macro-vascular invasion (CNLC IIIa)		11 (100%)
Treatment strategies	Donafenib alone	40 (63.5%)
	Combination treatment	23 (36.5%)
	Donafenib + ICI	14
	Donafenib + TACE	6
	Donafenib + ICI + TACE	3
Status of patients	Continued treatment	52 (82.5%)
Median duration of treatment (mDOT)	Continued treatment	5.5 months (IQR, 4.8–8.0)
	Discontinued treatment	4.0 months (IQR, 3.0–5.5)
Postoperative tumor recurrence		5 (7.9%)
	microvascular invasion (CNLC I-II)	11.1%
	macro-vascular invasion (CNLC IIIa)	18.2%
Adverse drug reactions (ADRs)	Any grade	34 (54.0%)
	Grade 3	4 (6.3%)

HC-25

A six and twelve classification-based nomogram for patients with hepatocellular carcinoma treated with transarterial chemoembolization

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Aims: The six and twelve (SAT) classification is a novel prognostic model for predicting survival in recommended TACE patients with hepatocellular carcinoma (HCC). However, a single parameter may compromise the accuracy and prediction of the model. We aimed to propose and validate an SAT-based prognostic nomogram for recommend-TACE patients. **Methods:** In this retrospective study, a total of 264 HCC patients receiving TACE for the first time were randomly assigned to the training set (n = 132) and the validation set (n = 132). A multivariate Cox proportional model for the training set was used to generate nomograms and an individual score (SATAH score). The predictive accuracy of the nomogram was evaluated by area under the curve, C-index and calibration tests. Its validity was also compared with the common model. **Results:** In multivariate analysis, SAT classification, serum α-fetoprotein level ≥ 400 ng/mL and serum HDL level were independently associated with the survival of patients in the training set. By summing the scores of these three prognostic predictors, a SATAH score from 0 to 160 was assigned to each patient. The nomogram generated from the training set had a concordance index of 0.750 (95% confidence interval [CI] 0.719–0.782), and the calibration plots consistently matched the ideal reference line for 1-, 2- and 3-year survival prediction. The discrimination test in the validation set provided a good concordance index of 0.641 (95% CI 0.603–0.679). Patients were grouped according to the SATAH score, and survival analysis was performed. The results suggested significant survival differences between the SATAH groups. The SATAH score and group had higher predictive efficacy than the other seven systems. **Conclusion:** SAT-based prognostic models can better identify the survival of recommend-TACE patients and may further aid in clinical decision-making. **Keywords:** liver cancer; TACE; nomogram; six and twelve; prognostic model.

HC-26

Effects of FOLFOX-HAIC and its combination therapy on unresectable hepatocellular carcinoma

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Aims: To explore the efficacy and safety of hepatic arterial infusion chemotherapy with FOLFOX regimen (FOLFOX-HAIC) combined with molecular targeted drugs and/or immune checkpoint inhibitors in unresectable hepatocellular carcinoma (HCC), and to provide a certain reference value for the selection of treatment options for unresectable HCC. **Methods:** The medical records of patients with unresectable hepatocellular carcinoma who received FOLFOX-HAIC treatment in the First Affiliated Hospital of Chongqing Medical University from June 2020 to December 2021 were retrospectively analyzed. To evaluate the tumor response, survival status, and adverse events after treatment. **Results:** A total of 69 patients were included, with a total of 225 HAIC treatments. The most common treatment regimen was HAIC combined with molecular targeted drugs and immune checkpoint inhibitors (76.8%). The overall objective response rate (ORR) was 53.6%, the disease control rate (DCR) was 85.5%, the median progression-free survival (PFS) was 9.23 months (95% confidence interval: 5.58-12.88 months), and the median overall survival (OS) not reached. 11.6% (8/69) of patients had tumor improvement after HAIC treatment and successful radical resection of liver cancer. In subgroup analysis, patients with HAIC combined molecular targeted drugs and immune checkpoint inhibitors regimens had an ORR significantly higher than the other treatment regimens (60.4% vs. 31.3%, P=0.041), and the median PFS was also significantly longer than the other regimens (12.23 vs. 4.5, P=0.014). No prognostic factors of OS were found in multivariate analysis, and HAIC combination treatment regimen was the only influencing factor of PFS. The incidence of adverse events of any grade was 95.7% (66 patients); the incidence of grade 3-4 adverse events was 31.9% (22 patients), mainly decreased platelet count (15.9%), decreased total number of white blood cells (7.2%), and increased AST. High (4.3%), abdominal pain (4.3%), elevated total bilirubin (2.9%), upper gastrointestinal bleeding (2.9%), and vomiting (1.4%). There were no treatment-related deaths. **Conclusion:** For unresectable hepatocellular carcinoma, FOLFOX-HAIC combined with molecular targeted drugs and immune checkpoint inhibitors has good therapeutic effects and acceptable related adverse effects, which is a safe and effective treatment option for patients with unresectable HCC. **Keywords:** hepatic arterial infusion chemotherapy; hepatocellular carcinoma; FOLFOX; molecular targeted drugs; immune checkpoint inhibitor.

HC-27

Efficacy and safety of envafolimab plus lenvatinib combined with TACE in unresectable hepatocellular carcinoma: An open-label, single-arm, phase 2 study (the CISLD-12 study)

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Aims: Transarterial chemoembolization (TACE) is one of the standard treatments for patients with intermediate-stage hepatocellular carcinoma (HCC). The combination of immune checkpoint inhibitors (ICIs) and lenvatinib has shown to be an effective regimen in patients with unresectable HCC (uHCC). In this study, we aim to investigate the efficacy and safety of combination of envafolimab (a novel, single-domain PD-L1 antibody) with lenvatinib plus TACE in uHCC patients. **Methods:** This is a prospective, open-label, single-arm, phase 2 study. Adults with confirmed uHCC with BCLC stage B or C, Eastern Cooperative Oncology Group performance status 0 or 1, Child–Pugh scored ≤7 and no previous systemic treatment for HCC are eligible. Patients will receive Lenvatinib once daily

plus envafolimab every 3 weeks plus TACE. TACE will be conducted repeatedly every 6 weeks on demand according to investigators' consideration, mainly based on the proportion of viable tumors. Imaging evaluation will be conducted every 6 weeks. The primary endpoint is objective response rate (ORR). Disease control rate (DCR), duration of response (DoR), progression free survival (PFS), overall survival (OS) and safety are evaluated as secondary endpoints (NCT05213221). **Results:** Thirty-nine patients were consecutively enrolled from March 14, 2022 to July 19, 2022. The median age was 57 years. At present, twenty-one patients were included for efficacy analysis, and twenty-nine for safety evaluation. Number of patients with BCLC stage B and C was 12 (57.1%) and 9 (42.9%), respectively. Six (28.6%) patients presented with portal vein tumor thrombus (PVTT) and 1 (4.8%) patient presented with hepatic vein tumor thrombus (HVTT). The ORR was 33.3% and 61.9%, and DCR was 85.7% and 85.7%, based on RECIST v1.1 and modified RECIST respectively. Seven (33.3%) patients reached the standard of conversion to resectable HCC and three (14.3%) patients received radical resection. The most common TRAEs were elevated AST (62.1%), elevated ALT (55.2%) and leukopenia (37.9%). Two (6.7%) patients experienced grade 4 toxicity (gastrointestinal bleeding and elevated ALT). No treatment-related deaths occurred. The survival data are not mature at present. **Conclusion:** Envafolimab plus Lenvatinib combined with TACE is a promising and tolerable therapeutic regimen for patients with BCLC B/C unresectable HCC. **Keywords:** hepatocellular carcinoma, Envafolimab, Lenvatinib, TACE.

HC-28

Thromboelastographic tracing of perioperative coagulation profile of 490 patients undergoing liver surgery and thromboelastography-guided management

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Aims: The liver itself is one component of the coagulation system. Alterations of hemostasis after liver surgery are complex and current evidence is confusing. Conventional coagulation tests generally provide limited information. Thromboelastography offers a comprehensive evaluation of the overall coagulation status. This study aims to define the perioperative coagulation profile of patients undergoing liver surgery by thromboelastography and to examine the effectiveness of thromboelastography-guided managements. **Methods:** A total of 490 patients with liver tumors were enrolled. Coagulation profile was evaluated by thromboelastography and conventional coagulation tests before operation and on postoperative days 1, 3, 5, 7 and 9. The perioperative coagulation managements were performed according to the thromboelastographic results. **Results:** At preoperative screening, majority of the patients (90.8%) showed normocoagulability. Of the patients with abnormal preoperative coagulation, hepatocellular carcinoma (HCC) patients were often associated with hypocoagulability due to thrombocytopenia derived from cirrhosis associated hypersplenism, whereas non-HCC patients mainly presented with hypercoagulability. After operation, thromboelastography showed normocoagulability in most patients (74.8%) despite conventional tests indicated prolonged prothrombin time. The incidence of hypercoagulability and hypocoagulability was 12.6% and 12.6%, respectively. Thromboelastography tracing revealed a considerable portion that conventional tests overdiagnosed. The postoperative coagulation profile was significantly correlated with severity of liver fibrosis/cirrhosis and blood loss. The patients with cirrhosis have a remarkably high incidence of hypocoagulability (16.1%) and low incidence of hypercoagulability (4.2%). The incidence of hypocoagulability in patients with mass blood loss (≥500 ml) reached 27.0% while that with minor blood loss was 9.7%. No patients experienced venous thromboembolism or bleeding. **Conclusion:** Our work reveals that normocoagulability is prevalent before and after liver surgery. Anticoagulant medication is not routinely required. However, the coagulation profile in patients undergoing liver surgery is complex and it is difficult to predict an individual's coagulation changes. Thromboelastography is recommended to be examined perioperatively and personalized treatment shall be determined accordingly. **Keywords:** liver neoplasm; liver surgery; coagulation; thromboelastography.

HC-29

Predictive value of gamma-glutamyl transferase to lymphocyte ratio in advanced hepatocellular carcinoma patients treated with anti-PD-1 therapy

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Aims: To assess the relationship between the gamma-glutamyl transferase to lymphocyte ratio (GLR) and survival outcomes in HCC patients who treated with immune checkpoint inhibitors (ICIs). **Methods:** From 9 May 2020 to 10 April 2022, the medical records of HCC patient who treated with immune checkpoint inhibitors (ICIs) were reviewed in Fudan Zhongshan hospital (Xiamen branch). The X-tile software was used to stratify the cut-off value of GLR. Survival outcomes between GLR <150 and ≥ 150 was analyzed by Kaplan-Meier curve with log-rank test, and risk factors for anti-programmed cell death protein 1 (anti-PD-1) immunotherapy were performed by univariable and multivariable Cox models. **Results:** A total of 106 patients were enrolled in this study. The mean follow-up time was 9.8 months (range: 1.7-28). The average age was 54 ± 11.8 years. Barcelona Clinic Liver Cancer stage B and C were 13(12.3%) and 93 (87.7%) patients, respectively. GLR <150 was associated with longer overall survival (OS) compared with GLR ≥ 150 in baseline (21.3 vs 7.4 months, $P < 0.001$). By multivariate analysis, GLR <150 was strongly considered as an independent prognostic factor for better survival (HR 2.56, $P = 0.02$). **Conclusion:** This study supports that GLR <150 in the pretreatment setting predicted better OS in HCC patients with anti–PD-1 therapy and need to be conformed by a larger cohort. **Keywords:** gamma-glutamyl transferase to lymphocyte ratio (GLR); Hepatocellular carcinoma; overall survival; prediction.

HC-30

Efficacy analysis of comprehensive therapy of immune checkpoint inhibitors (ICIs) and tyrosine kinase inhibitors (TKIs) combined with HAIC or TACE in unresectable hepatocellular carcinoma (uHCC)

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Aims: From August 2020 to July 2022, patients who received HAIC or TACE combined with ICIs and TKIs were retrospectively analyzed. Treatment-related adverse events (AEs) were recorded. The Kaplan–Meier method was used to estimate time to progression (TTP) and progression-free survival (PFS).

Method: From August 2020 to July 2022, patients who received HAIC or TACE combined with ICIs and TKIs were retrospectively analyzed. Treatment-related adverse events (AEs) were recorded. The Kaplan–Meier method was used to estimate time to progression (TTP) and progression-free survival (PFS). **Results:** The last evaluation time of 44 patients in this group was July 2022. In total, 44 patients with uHCC were included. according to mRECIST. The objective response rate (ORR) and disease control rate (DCR) were 52.27% and 79.5%.The success rate of image-based transformation was 20.45% (9/44), and the actual transformation resection rate was 18.18% (8/44).There were no adverse events of grade 3-4 or above in the remission group. Two grade 3-4 adverse events occurred in the non-remission group. There were 44 patients in this group, 23 in the remission group and 21 in the non-remission group. The follow-up time was 1-20 months, and the median follow-up time was 8 months. During the follow-up period, 13 patients had tumor progression or recurrence. Only 1 patient died of postoperative recurrence. **Conclusion:** Checkpoint inhibitors (ICIs) and tyrosine kinase inhibitors (TKIs) for the treatment of unresectable hepatocellular carcinoma (uHCC) is safe. Triple therapy can achieve satisfactory conversion resection rate of unresectable HCC, which will provide a new treatment strategy for it. At the same time, for CR patients who respond, generally after 2-3 intervention cycles, surgery should be performed as soon as possible. For PD patients, continuous interventional therapy is still ineffective, and systemic therapy should be used instead of continuous interventional therapy, which reduce the burden on the liver. **Keywords:** ICIs; TKIs; HAIC; TACE; uHCC.

HC-31

The prognostic role of HBV-DNA in unresectable hepatocellular carcinoma undergoing immune checkpoint inhibitor plus multityrosine kinase inhibitor: a real-world study

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Aims: To investigate the prognostic effect of Hepatitis B virus (HBV) in unresectable hepatocellular carcinoma (uHCC) treated with immune checkpoint inhibitor (ICI) plus multityrosine kinase (TKI) in real-world study. **Methods:** This retrospective study included 175 patients with HBV-related uHCC undergoing ICI plus TKI treatment at Zhongshan Hospital (Xiamen), Fudan University from January 2019 to December 2021. Baseline features and subsequent follow-up data were collected. Efficacy was assessed within patients who finished at least two imaging examinations. Univariate and multivariate analysis of risk factors were conducted using Kaplan-Meier curves and Cox regression model. **Results:** In total, 101 patients were enrolled with median follow-up duration of 11.68 months, median progression-free survival of 7.07 months and median overall survival (OS) of 17.57 months. Among them, 68 patients had the decreased level of hepatitis B surface antigen and 15 patients had increased level of HBV-DNA at 12 weeks compared with the baseline. Besides, 5 patients showed HBV reactivation and only one of them was accompanied with hepatic function failure. Patients with elevated level of HBV-DNA had a shorter median survival time (7.86 months vs. 12.40 months, $p = 0.006$, log-rank test). Furthermore, univariate and multivariate analysis showed that elevation in HBV-DNA level at 12 weeks after treatment was an independent predictive factor for poor survival in HBV-related uHCC. **Conclusion:** The results suggested that patients with HBV-related uHCC could have survival benefit from combinational treatment of ICI and TKI. Elevation of HBV-DNA level at 12 weeks after treatment may enable to independently predict poor outcome for patients with HBV-related uHCC. **Keywords:** hepatocellular carcinoma; immune checkpoint inhibitor; multityrosine kinase inhibitor; hepatitis B virus; prognosis.

HC-32

Analysis of myocardial injury caused by targeted therapy combined with immune checkpoint inhibitor in the treatment of hepatocellular carcinoma

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Aims: To analyze the incidence and clinical characteristics of myocardial injury caused by targeted therapy combined with immune checkpoint inhibitor in the treatment of hepatocellular carcinoma. **Methods:** 125 patients with hepatocellular carcinoma who received targeted immunotherapy in Department of Hepatic Oncology, Xiamen Clinical Research Center for Cancer Therapy, Zhongshan Hospital, Fudan University (Xiamen Branch) from January 2017 to December 2021 were collected. According to the changes of cardiac troponin T (cTnT) level during treatment, they were divided into cTnT normal group and cTnT elevated group. The baseline characteristics of the two groups and the clinical changes of cTnT elevated group before and after treatment were analyzed. **Results:** In targeted immunotherapy for hepatocellular carcinoma, the incidence of myocardial injury was 8.8% (1/125), of which the incidence of myocarditis was 0.8% (1/125). Elderly patients, cardiopathy, diabetes may be a risk factor for the risk of myocardial injury in targeted immunotherapy for hepatocellular carcinoma. During treatment, the decrease of left ventricular ejection fraction, the decrease of left ventricular end diastolic diameter and the increase of interleukin-2 receptor were associated with

myocardial injury. **Conclusion:** The incidence of myocardial injury is not low in targeted immunotherapy for hepatocellular carcinoma. Follow-up and vigilance are needed to avoid serious adverse events. **Keywords:** hepatocellular carcinoma; targeted therapy; immunotherapy; myocardial injury.

HC-33

The immune-metabolic crosstalk between CD3+C1q+TAM and CD8+T cells associated with relapse-free survival in HCC

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Aims: Hepatocellular carcinoma (HCC) is the most prevalent form of liver cancer with high mortality. Understanding HCC immune microenvironment (TME) is critical for exploring novel treatment strategy. Recently studies have portraited multidimensional profiling of HCC microenvironment by using single-cell RNA sequencing (scRNA-seq). Still, few focus on the roles of cell-cell interaction and immunometabolic regulation. **Methods:** We carried out scRNA-Seq of CD3+ tumor-infiltrating lymphocytes sorted from the paired tumor and peri-tumor tissues of HCC patients. The cluster interaction scores were calculated based on scRNA-seq data via Cellphone DB. The scVelo and CytoTRACE analyses were conducted to explore differentiation trajectory. Besides, we performed survival analysis based on TCGA to access the predictive value of identified clusters. Furthermore, developing in vitro T cell culture model to validate the interactions between specific cell subsets. **Results:** A total of 19 immune cell clusters were identified, and several were found related to HCC prognosis. Differentiation trajectory suggested that the differentiation potential of CD4+ and CD8+ T cell subsets from high to low. Besides, a previously unreported population of C1q+CD3+tumor-associated macrophages (TAM) was detected and found significantly interplayed with CCL4+CD8+T cells. Compared with peri-tumor tissue, their interaction was attenuated in the tumor. Furthermore, we found that C1q+CD3+TAMs affected T cell immunity through C1q signaling-induced metabolic and epigenetic remodeling, potentially affecting tumor prognosis.The dynamic presence of C1q+CD3+macrophages were also verified in peripheral blood of clinical sepsis samples. **Conclusion:** Collectively, our findings unveil the immune-metabolic crosstalk between CD3+C1q+TAMs and CCL4+CD8+T cells, which may provide a novel strategy for regulating immunosuppressive TME and enhancing immunotherapy in HCC. **Keywords:** HCC; immunometabolism; C1q; tumor-associated macrophage; T cell.

HCC-non-clinical

HNC-01

ID1/Myc signaling drives immune evasion through PD-L1 upregulation and polymorphonuclear myeloid-derived suppressor cells recruitment in oxaliplatin-resistant hepatocellular carcinoma

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Aims: Previously, we have demonstrated ID1/Myc signaling is highly expressed in oxaliplatin-resistant hepatocellular carcinoma (HCC). This study sought to investigate the role of ID1/Myc signaling on immune evasion in oxaliplatin-resistant HCC. **Methods:** The oxaliplatin-resistant hepatocellular carcinoma cell lines (Hepa 1-6-OXA, 97H-OXA and 3B-OXA) were established and their oxaliplatin tolerance was confirmed in vitro and in vivo. The relationship between ID1/Myc and PD-L1 upregulation, polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) accumulation was explored. The underlying mechanism in which ID1/Myc signaling regulated PD-L1 expression and PMN-MDSCs accumulation was investigated in vitro and vivo. **Results:** Increased ID1/Myc expressions were identified in oxaliplatin-resistant HCC and correlate with PD-L1 upregulation and PMN-MDSCs accumulation. The knockdown of Myc sensitized oxaliplatin-resistant HCC cells to oxaliplatin and resulted in a decrease of PMN-MDSCs and an increase of IFN-γ+ CD8+ T cells in tumor microenvironment. PCR array, ELISA assay and MDSC transwell migration assay indicated that oxaliplatin-resistant HCC cells recruited PMN-MDSC through CCL5. The Dual luciferase reporter assay indicated that Myc could directly increase the transcriptions of PD-L1 and CCL5. Furthermore, anti-PD-L1 antibody combined with CCL-5 blockade exhibited significant anti-tumor effects in oxaliplatin-resistant HCC. **Conclusion:** ID1/Myc signaling drives immune evasion in oxaliplatin-resistant HCC via PD-L1 upregulation and PMN-MDSCs recruitment. Blocking the ID1/Myc pathway represents a promising treatment target to conquer immune escape as well as chemoresistance in HCC. **Keywords:** hepatocellular carcinoma; chemoresistance; immune evasion; polymorphonuclear myeloid-derived suppressor cell; PD-L1.

HNC-02

Two molecularly distinct subtypes and immunosuppressive microenvironment of keratin 19-positive cancer stem cells in HBV-associated hepatocellular carcinoma based on scRNA-seq

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Aims: The over-expression of Keratin 19 (CK19) was an important poor prognostic marker for hepatocellular carcinoma (HCC) and was incorporated into various HCC molecular typing systems. However, the understanding of CK19+CSC classification and immune microenvironment in HBV-associated HCC is limited. **Methods:** Tissue samples were collected from 11 patients with HBV-related HCC (discovery cohort), including 7 CK19-positive, 3 CK19-negative tumor tissues and 5 adjacent tissues. Then scRNA-seq experiments were performed using the Singleron platform. Surgically resected tumor tissue and paired para-cancerous tissue samples from 96 patients with HBV-related HCC were collected for bulk RNA sequencing and their clinicopathological data were recorded (validation cohort 1). HCC cell lines (HCC_97L, HCC_LM3) were selected to construct a stem cell-enriched nude mouse model. We selected the normal liver cell line LO2 with low CK19 expression, the cholangiocarcinoma cell line HCCC9810 with significantly high CK19 expression, and hepatocellular carcinoma with relatively high CK19 expression (HUH7, SNU182, HCC_97 and HCC_LM3), as well as HCC_97L and HCC_LM3 primary nude mouse tumorigenic tissues (Tissue_97 and Tissue_LM3) to conduct scRNA-seq as validation cohort 2. In addition, the published spatial transcriptome data of CK19+HCC patient tissues (including tumor and non-tumor) was used to uncover the spatial distribution of TAM_SPP1. The results of the analyses for scRNA-seq data were validated using validation cohort 1 or 2, Spatial transcriptome, immunohistochemistry or multiplex immunofluorescence and in vitro and in vivo experiments. **Results:** In this study, we sequenced a total of 64,581 single cells derived from human HCC and adjacent tissues. We delineated the transcriptome profile of human CK19+ HCC, which is classified into 11 major cell types, including cancer cells, immune cells, and stromal cells. First, we found CK19+CSCs co-express multiple consensus markers of CSCs in malignant cells, such as EPCAM, PROM1, SOX9, CD24, ALDH1A1, DLK1 and LGR5. Then, the heterogeneity of malignant cells in CK19+ HCC patients was revealed at the single-cell level and two molecularly distinct cellular subtypes were found in CK19-positive CSCs, verified by validation cohort 2. The foetal hepatocyte subtype regulated by the MYC transcription factor has strong stemness characteristics. The adult HHyP subtype, which is regulated by SOX9, possesses stronger metastatic ability and worse prognosis. CLDN4 was identified as a marker that differentiates the two cellular subtypes of CK19+CSC. Moreover, myeloid-derived cells were classified into 7 categories in the discovery cohort. The immune microenvironment of suppressive SPP1-positive tumor-associated macrophages (TAM_SPP1) in CK19+ HCC is further elucidated. We found that CK19+ HCCs were significantly enriched in SPP1-TAM subset cells compared with CK19-negative HCCs. TAM_ SPP1 promoted tumor invasion and metastasis at the tumor invasive front of CK19+ HCC by activating angiogenesis, EMT, hypoxia and KRAS signaling pathways. And patients with high TAM_SPP1 enrichment had shorter overall survival and disease-free survival. In addition, we further disclosed the microenvironment of T/NK cell immune exhaustion. Relative to non-tumor CD8_GZMH, CD8_NR4A1, NK_CD160 and NK_CD16 cell subsets were significantly reduced and T_MKI67 was significantly enriched in CK19+HCC, whereas only NK_CD160 was reduced in CK19-negative HCC. The cytotoxic CD8_GZMH was significantly reduced in CK19+HCC compared to CK19-HCC. We further found that T_MKI67 expresses multiple immune checkpoint markers, such as HAVCR2, TIGIT, CTLA4, LAG3 and PDCD1, verified by validation cohort 1. **Conclusion:** Our findings provide a comprehensive transcriptomic landscape of human CK19+HCC at single-cell resolution. Stem-strong foetal hepatocyte and high-metastatic adult HHyP subtypes were identified in CK19+CSC, with distinct regulatory mechanisms in these subtypes. Enrichment of SPP1-TAM and T_MKI67 were found in CK19+HCC. These findings provide the basis for effective immuno-oncology therapy in HCC. **Keywords:** hepatocellular carcinoma; cancer stem cell; keratin 19; single-cell transcriptome sequencing; immunosuppressive microenvironment.

HNC-03

CRISPR-engineered culturable bipotent progenitor cells induced hepatocarcinogenesis

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Aims: Hepatocellular carcinoma (HCC) is a common malignancy which is often diagnosed at an advanced stage and has a poor prognosis. A better understanding of the development of HCC could provide potential preventive therapies and improve patient survival. Currently, models to study hepatocarcinogenesis are largely missing. Here, we established a new model of hepatocellular carcinoma using CRISPR-engineered liver progenitor cells. **Methods:** Mouse mature hepatocytes (mMHs) were obtained utilizing a perfusion method. Using a combination of three small molecules, A-83-01, CHIR99021 and Y-27632, mMHs were reprogrammed into chemically induced liver progenitors (CLiPs) in vitro. Finally, the CLiPs were genetically modified based on the molecular scissors CRISPR/Cas9. **Results:** In this study, based on small molecule reprogramming techniques, we reprogrammed mouse MHs into chemically induced liver progenitor cells using a combination of three small molecules, A-83-01, CHIR99021 and Y-27632 in vitro. The expression of various hepatocyte-specific genes was found in CLiPs. In addition, we developed a plasmid toolbox that integrates the expression of the proto-oncogenes NRAS and c-MYC on the top of a plasmid that mediates the expression of P53 sgRNA. Using this toolbox, we performed a gene editing of liver histocytes in vitro. The results showed that after knockout of P53 and introduction of oncogenes NRAS and c-MYC, CLips were reprogrammed to transform into hepatocellular carcinoma cells, which gradually assumed the structure and function of HCC in vitro. Finally, through in vivo experiments, we confirmed that the edited CLips could form HCC. **Conclusion:** Our study provides a new model to study HCC genesis in vitro. By editing

different tumor suppressor genes and introducing different proto-oncogenes, or adding microenvironmental factors such as immune cells, stromal cells, etc., this model can be used to explore molecular changes in HCC development and new therapies for preclinical liver cancer. **Keywords:** CRISPR/Cas9; small molecule reprogramming; liver progenitor cells; hepatocellular carcinoma.

HNC-04

PARP inhibitors contribute to radiation induced primary and abscopal tumor control by activating innate immune response

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Aims: Poly (ADP-ribose) polymerase (PARP) inhibitors have been clinically used in cancers with BRCA mutation and been under investigation for their ability to enhance responses to radiotherapy. However, a poorly understood feature of these responses is PARP inhibitors potentiates the sensitivity to radiotherapy in BRCA-proficient cells. Herein, we intend to investigate whether olaparib sensitizes hepatocellular carcinoma (HCC) cells to radiation. **Methods:** HCC cells were treated with irradiation with or without olaparib and DNA damage and cell proliferation were analyzed. Immune deficient, immune competent, and cGAS knockout mice underwent X-ray irradiation of 24 Gy in three fractions were used to investigate the roles of olaparib, irradiation and anti-CTLA4 on tumor growth and tumor microenvironment. **Results:** We show that a low dose of olaparib sensitized HCC cells to radiation. Olaparib and irradiation coadministration resulted in severe DNA damage by producing double-strand breaks (DSBs) as revealed in vitro and in immune-deficient mice. Moreover, double-strand DNA activating cGAS-STING signaling, contributed to antitumor innate immunity in both irradiated and abscopal tumors. Olaparib further enhanced radiotherapy-stimulated CD8+ T cell infiltration in xenografts. Mechanism study revealed that the activation of cGAS-STING and type I interferon production potentiates the T cell priming against tumor neoantigens induced by irradiation. Furthermore, olaparib attenuated immune exhaustion of radiation and contributes to abscopal tumor control of HCC to immune checkpoint inhibitors. **Conclusion:** Combination therapy with PARP inhibitors and radiotherapy may contributes to local (primary) and systemic (abscopal) tumor control and enhance responsiveness of HCC to immunotherapy. **Keywords:** irradiation; olaparib; abscopal effect; STING signaling; innate immune response.

HNC-05

Deciphering tumor ecosystem following immune checkpoint blockades in hepatocellular carcinoma via analyzing a public single-cell dataset

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Aims: Immune checkpoint blockades (ICBs) based systemic treatment regimens have demonstrated promising anti-tumor efficacy in hepatocellular carcinoma (HCC), while the specific mechanisms underlying their responsiveness or non-responsiveness remain elusive. In this study, we aimed to decipher the intra-tumor changes in response to ICBs in HCC at a single-cell resolution via analyzing a publicly available single cell dataset. **Methods:** Single-cell RNA sequencing (scRNA-seq) dataset (GSE151530) of patients with liver cancer enrolled for ICBs clinical trial were downloaded from Gene Expression Omnibus (GEO). We extracted and systematically re-analyzed 25 HCC biopsies from 18 patients, with 12 biopsies collected before and after treatment form 5 patients. The Seurat v4 R package was used to analyze the scRNA-seq data. Cell type annotation was performed manually according to highly expressed marker genes. CellChat R package was utilized to analyze intercellular communication and differential ligand-receptor interactions. Comparisons of gene signature scores between two groups of cells were performed using unpaired two-tailed Wilcoxon rank-sum tests. Statistical analyses were performed using R version 4.1.2 and statistical significance was defined as p < 0.05. **Results:** Among the 25 biopsies, 16 were collected at baseline, and 9 were collected at different time points following ICBs therapy. Single-cell transcriptomic data of 26,068 cells were obtained, with 19442 cells from treatment-naïve biopsies (designated as P-HCC) and 6626 cells from ICBs-treated group (denoted as T-HCC). Six major cell types were defined after clustering analysis: B cells, cancer-associated fibroblasts (CAFs), Malignant cell, T cells, tumor-associated macrophages (TAMs) and tumor-associated endothelial cells (TECs). The cell type ratios were comparable between baseline and ICBs treatment groups. Differentially expressed gene (DEG) analysis of malignant cells suggested an enrichment of genes involved in metabolism-related pathways in T-HCC, whereas genes upregulated in P-HCC were mainly involved in canonical oncogenic pathways. Re-clustering analysis of T cells revealed 11 subpopulations, including 6 subtypes of CD4+ T cell, 4 clusters of CD8+ T cells and cycling T cells. Cellular proportion analysis revealed that CD4_CCR7_naive cells were mainly enriched in P-HCC. While the CD8 T cell subtypes showed no significantly different distribution between P-HCC and T-HCC samples, CD8_GZMK cells, which highly expressed cytotoxic genes (GZMK, GZMA, and NKG7) and moderately expressed exhaustion-related markers (TIGIT, PDCD1, and LAG3), displayed a rising trend of proportion in samples treated with ICBs compared with that in baseline samples. Finally, we performed differential analysis of ligand-receptor (L-R) pairs between malignant

cells and other immune cell types. Strikingly, we found an enhanced SPP1 signaling mediated by L-R pair SPP1-CD44 in T-HCC, initiating from malignant cells and targeting immune cells including B cells, T cells and TAMs. This finding was consistent with the upregulated SPP1 expression in malignant cells following ICBs, suggesting that this signaling plays a pivotal role in promoting tumor evolution following ICBs therapy. **Conclusion:** Our single-cell analyses unveil the altered landscape in HCC tumor ecosystem treated with ICBs. A rising trend of proportion of the cytotoxic CD8_GZMK cells suggests that ICBs may correct and reinvigorate the tumor-killing response of this CD8 subcluster against tumor. Our study also highlights the significance of targeting SPP1-CD44 signaling to overcome ICBs resistance and develop more effective ICBs based therapy for HCC. **Keywords:** tumor ecosystem; Immunotherapy; HCC; Single-cell analysis; Data mining.

HNC-06

Genome-scale CRISPR screen identifies LAPTM5 driving lenvatinib resistance in hepatocellular carcinoma

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Aims: Drug resistance has greatly limited the clinical efficacy of Lenvatinib in hepatocellular carcinoma (HCC). This study was aimed to identify potential targets that act synergistically with Lenvatinib and reveal its underlying mechanisms. **Methods:** Integrated unbiased whole-genome CRISPR/Cas9 screen with database analysis were performed to define genes potentially driving Lenvatinib resistance. Combined in vitro and in vivo assays were implemented to confirm the critical role and underlying mechanisms of lysosomal protein transmembrane 5 (LAPTM5) in Lenvatinib resistance. Multiple HCC models were applied to validate the clinical significance of LAPTM5. **Results:** Combination of whole-genome CRISPR/Cas9 screen with database analysis indicated LAPTM5 as the critical contributor to Lenvatinib resistance in HCC. We revealed that LAPTM5 could promote intrinsic autophagic influx by facilitating autolysosome formation to drive Lenvatinib resistance. The upregulation of LAPTM5 in HCC was induced by both DNA hypomethylation and driver mutations like TP53. Inhibition of autolysosome formation by either Hydroxychloroquine (CQ) or LAPTM5 abrogation worked synergistically with Lenvatinib to inhibit tumor growth. In HCC cell lines, patient derived primary cell lines and organoids, as well as human HCC xenografts and immunocompetent mouse HCC model, the close association between LAPTM5 and sensitivity to Lenvatinib was consistently verified. Importantly, in clinical HCC samples, where Lenvatinib was used as the first line or adjuvant therapy, LAPTM5 expression positively correlated with Lenvatinib sensitivity, implying it as a biomarker to predict patient response to Lenvatinib. **Conclusion:** Our results showed that the combinational therapy targeting autophagy represented a promising strategy to overcome Lenvatinib resistance in HCC, and LAPTM5 expression could provide potential guidance for clinical interference. **Keywords:** HCC; Lenvatinib; LAPTM5; resistance; autophagy; whole-genome CRISPR/Cas9 screen.

HNC-07

The role of tumor-infiltrating B cells in the tumor microenvironment of hepatocellular carcinoma and its prognostic value

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Aims: Unveil the role of tumor infiltrated B cells in the microenvironment of hepatocellular carcinoma. **Methods:** We conducted an integrative analysis of scRNA-seq datasets in HCC tumor samples. We analyzed the features of B cells in normal liver tissue and hepatocellular carcinoma. Additionally, we conducted a deconvolution analysis using the matrix of scRNA-seq datasets and the RNA-seq datasets in TCGA-LIHC database. Finally, we performed immunohistochemistry analysis of primary tumor tissue of HCC patients using antibodies against CD79A and validated the impact of tumor infiltrated B cells in the prognosis of LIHC. **Results:** We identified several subtypes of B cells in the microenvironment of HCC, including the plasma cells and naïve B cells. The relative ratio of B cells, but not the plasma cells, was significantly decreased in HCC as compared to the normal liver tissue. In addition, genes related to antigen-presenting and cell proliferation were decreased in tumor-infiltrated B cells. The observation of B cell infiltration was further validated with the TCGA-LIHC cohort. Moreover, we found higher infiltration of B cells was associated with a favorable outcome of LIHC in both the TCGA-LIHC cohort and our cohort. **Conclusion:** The tumor infiltrated B cells potentially exert tumor-suppressive function in the microenvironment of HCC and the higher levels of B cell infiltration were associated with a favorable outcome of HCC. **Keywords:** hepatocellular carcinoma; tumor microenvironment; B cells; prognosis.

HNC-08

SIGLEC15 was negatively correlated with PD-L1 in hepatocellular carcinoma and could induce cytotoxic T cell apoptosis through ANXA2/pERK/NFkB axis

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Aims: The functional roles of SIGLEC15 in immune evasion of hepatocellular carcinoma (HCC) were not clear, and investigation of SIGLEC15 could provide new ideas for tumor immune therapy. **Methods:** The expression levels of SIGLEC15 and PD-L1 were examined in HCC tissues to explore their associations with clinical factors. SIGLEC15 was knocked down or overexpressed in cell lines to explore its roles in tumorigenesis in vitro and in vivo, using T cell-deficient (BALBc nude) and immunocompetent (C57BL) mice. Co-culture system of Jurkat and tumor cell lines and SIGLEC15 blockade antibody were used to explore the influences of SIGLEC15 on T cells in the tumor microenvironment. Mononuclear phagocyte depletion was performed by using anionic clophosome. Flow-cytometry analysis was performed to examine T cell functions, as well as apoptotic and proliferative status. Bulk and single-cell sequencing analyses, as well as cytokine microarray analysis, were performed to investigate T cell status. Human

protein array, protein pull-down, western blot, genetic truncation, mass spectrometry and molecular docking analyses were used to locate SIGLEC15-interacting proteins and regulating pathways. **Results:** SIGLEC15 expression was increased in HCC tumor tissues and was negatively correlated with PD-L1 expression. SIGLEC15 knock-down or blockade suppressed tumor growth by promoting CD8+ T cell proliferation and cytotoxic function. SIGLEC15 could interact with ANXA2 to induce CD8+ T cell apoptosis through pERK/NFkB axis. **Conclusion:** Tumor-derived SIGLEC15 could induce T cell apoptosis through ANXA2/pERK/NFkB axis and was negatively correlated with PD-L1 in HCC, indicating its potential as a target for PD-L1-negative patients. **Keywords:** immune evasion; tumor microenvironment; immune checkpoints; liver cancer.

HNC-09

Glutathione peroxidase 8 suppressed hepatocellular carcinoma (HCC) stemness and metastasis potential through Hsc70/AKT axis

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Aims: We found that the GPX8 protein abundance in HCC tissues was significantly lower compared with adjacent tissues, and its low expression was related to poor prognosis. However, roles of GPX8 in HCC development have not been systematically investigated, and the current study remain controversial. Here, we investigated the roles of GPX8 in tumor stemness of HCC. **Methods:** The mRNA expression level of GPX8 in TCGA-LIHC, prognosis and correlation with other genes were investigated by GEPIA (http://gepia.cancer-pku.cn/). Immunohistochemical staining was used to detect the protein expression level of GPX8 in 354 cases of HCC tissue microarray, and the correlation between GPX8 and clinical features, survival and recurrence was analyzed to evaluate the value of GPX8 as a clinical prognostic marker. The sphere formation array, plate cloning-formation assay and cell migration assay were used to evaluate the stemness and migration ability of HCC cells affected by GPX8 down-regulation. Nucleus-cytoplasmic separation and immunohistochemical staining were used to identify the subcellular location of Hsc70. The Hsc70 small interfering RNA was used to explore whether the regulation of PI3K p110 subunit expression and AKT phosphorylation by GPX8 down-regulation was dependent on Hsc70. **Results:** The GPX8 protein level was downregulated in HCC and the low expression predicted early recurrence and poor prognosis in HCC patients. Downregulation of GPX8 promoted stemness and metastasis of HCC cells through activation of AKT signaling pathway. There was an interaction between GPX8 and Hsc70, and down-regulation of GPX8 could reduce the interaction with Hsc70 and promote its nuclear translocation. Downregulation of GPX8 could promote the expression of PI3K p110 subunit, but this effect was dependent on Hsc70, and knockdown of Hsc70 could reverse the phosphorylation of AKT induced by GPX8 down-regulation. There was a significant negative correlation between the protein level of GPX8 and the nuclear positive rate of Hsc70 and the expression level of KLF4 in clinical HCC patients. **Conclusion:** Our results indicate the antitumor function of GPX8 in HCC by inactivation of the Hsc70/AKT pathway, which may serve as a potential target for HCC therapy. **Keywords:** HCC; glutathione peroxidase 8 (GPX 8); Hsc70; AKT.

HNC-10

Investigation of the immune-modulating effect of MLKL in hepatocellular carcinoma

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Aims: Necroptosis is a newly discovered form of programmed cell death that is strictly regulated by pathways and is performed by MLKL. It is suggested that necroptosis may be related to the cancer development. However, studies on necroptosis in tumors are rare and the results are controversial. Therefore, we took liver cancer as the research system to explore the effects of necroptosis on tumor and its immune microenvironment. **Methods:** RNAi, CRISPR/Cas9, western blot, FACS. **Results:** To identity the role of MLKL in cancer, we analyzed the tissue microarray and found that MLKL expression in liver cancer tissues is significantly higher than that in adjacent tissues. More importantly, its high expression is correlated with a poor prognosis. Thus, we used RNAi technique to knock down the expression of MLKL in PDCs from liver cancer and studied its effect on cell proliferation, migration. However, down-regulation of MLKL expression had no effect on cell proliferation or cell migration. Given the pro-inflammatory response of necroptosis, we then speculated MLKL might affects the tumor growth via regulating tumor microenvironment. In order to do this, a MLKL-knockout hepa1-6 cell line was constructed using the CRISPR/Cas9 system and C57BL/6 mice were orthotopically implanted with MLKL-knockout hepa1-6 cell(MLKL-KO) and control cells(MLKL-MOCK). Inconsistent with our previous result, knockout MLKL expression in Hepa1-6 led to a significantly restricted tumor growth in this murine tumor model. Next, the tumor-infiltrating immune cells were then analyzed. Compared with tumors from MLKL-MOCK mice, tumors from MLKL-KO mice were totally different in the composition and activation state of the immune infiltrate. We found MLKL-KO tumors exhibited significantly increased the frequency of total hematopoietic cells (CD45+) and infiltration of lymphoid cell populations, such as CD8+ cytotoxic T cells, natural killer (NK) cells, and significantly decreased infiltration of myeloid cell populations, such as myeloid-derived suppressor cells (MDSCs), indicating that MLKL deletion enabled to reprogram a panel of TME immune cells and which was a perfect and important system to increase the therapeutic efficacy of cancer immunotherapy. Therefore, we predicted the anti-PD-1 antibody should be effective in this case. In agreement with our prediction, anti–PD-1 led to significant tumor inhibition in MLKL-KO Hepa1-6 tumors, but caused almost no change in MLKL-MOCK tumors. Together, these results demonstrated that anti–PD-1 immunotherapy was effective against MLKL-KO mouse Hepa1-6 tumors, which was associated with a remodeling of antitumor immunity. Given an important characteristic of cytokines is the ability to interact with the tumor cells and tumor microenvironment, we speculate that cytokines might also be involved in the immunomodulation mechanism of MLKL.

To better understanding the impact of MLKL on tumor immunomodulation, we performed cytokine arrays analysis of MLKL-mock and MLKL-KO tumor tissue homogenate. The data revealed MLKL knockout caused significant changes in cytokine levels. These cytokines regulated the function of immune cells previously studied. These results suggested that the immunomodulation of MLKL in tumor progression was upregulated immune-activating cytokines and downregulated immune-suppressive cytokines, which was consistent with previous study. Thus, to further clarify the molecular mechanism underlying regulation of MLKL immunomodulation, KEGG enrichment analysis was performed in differentially expressed cytokines. The pathway enrichment analysis of these cytokines uncovered JAK-STAT pathway had a high level of involvement. Further, we found depletion of MLKL might lead to activate JAK-STAT signaling as a potential mechanism to account for the reconstitution of TEM. **Conclusion:** To sum up, our findings underscore two key facts: (i) the importance of targeting protein complex in cancers cells that enable to suppress tumor immune landscape via reprogramming the cytokine repertoire and (ii) that MLKL is a fundamental driver of immune suppressing phenotype in TME. Insight knowledge of the basic mechanisms of MLKL responsible for reconstitution of TME has enabled us to draw a more comprehensive picture of the possible points of intervention and has provided us with reasons that might account for immune therapeutic failure. **Keywords:** necroptosis; MLKL; cytokine; immunotherapy.

HNC-11

SH2 domain-containing protein tyrosine phosphatase 2 inhibition potentiates PD-1 inhibition in hepatocellular carcinoma

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Aims: Despite the treatment of HCC has been revolutionized by the advent of effective systemic therapies. There is an unmet need to develop a novel strategy for HCC treatment due to the poor prognosis. **Methods:** The expression of SH2 domain-containing protein tyrosine phosphatase 2 in HCC tissues and cell lines was detected by qRT-PCR and western blot. Clinical significance of SH2 domain-containing protein tyrosine phosphatase 2 was evaluated by Cox regression analysis. The role of SH2 domain-containing protein tyrosine phosphatase 2 in HCC progression was assessed by transwell assays, CCK-8, clony formation assays, flow cytometry in vitro. Orthotopic xenograft tumor modoel and using both NOD/SCID mice and C57BL/6 mice was adopted to investigate functions of SH2 domain-containing protein tyrosine phosphatase 2 in vivo. **Results:** SH2 domain-containing protein tyrosine phosphatase 2, which is elevated in most HCC tissues, correlated with poor survival of HCC patients. Functional experiments reveals the oncogenic role of SH2 domain-containing protein tyrosine phosphatase 2, which promotes HCC growth and metastasis through c-Jun-dependent modulation of interferon signaling. Moreover, SH2 domain-containing protein tyrosine phosphatase 2 inhibition promotes multiple pro-inflammatory cytokines such as IFN-gama, TNF- alpha release and recruites NK cells into the tumor microenvironment. We have also demonstrated that SH2 domain-containing protein tyrosine phosphatase 2 inhibition could act in synergy with anti-PD-1 antibody in HCC murine model as well as HCC organoids. **Conclusion:** Overall, we explored for the first time targeting SH2 domain-containing protein tyrosine phosphatase 2 synergizes with anti-PD-1 antibody in preclinical models of HCC. **Keywords:** HCC; SH2 domain-containing protein tyrosine phosphatase 2; PD-1.

HNC-12

Integrated genomic and transcriptomic profiling to dissect the spatiotemporal evolution of metastatic hepatocellular carcinoma

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Aims: As highly heterogeneous primary lesions and the presumably more homogenous metastatic tumors evolve, a comprehensive genetic and molecular landscape of metastatic disease by sequencing multiple-regional samples from matched primary and metastatic tumors is desperately needed to clarify the biologic underpinnings of metastatic progress and identify more effective therapeutic strategies for advanced HCC. **Methods:** A total of 501 formalin-fixed, paraffin-embedded (FFPE) tumor samples were collected from 224 HCC patients. The discovery cohort was comprised of two distinct clinical outcomes: the extrahepatic metastasis group included paired primary tumors (PTs), intrahepatic relapses (RTs) and extrahepatic metastases (MTs) (METS group), and no relapse/metastasis group of patients with long-term no relapse or metastasis after surgery (progression-free group). The validation cohort included 88 patients with unpaired primary tumors of different clinical outcomes. All samples were profiled using whole-exome sequencing (WES) with an average coverage of 170× on the exon regions and RNA sequencing (RNA-seq). Among them, 52% of PTs and MTs in the METS group of the discovery cohort were multi-regionally sequenced. Digital pathology and digital spatial profiling (DSP) of primary, intrahepatic recurrent, and extrahepatic metastatic tumors from HCC patients in our cohort were also performed. **Results:** We first performed calling of single nucleotide variants (SNVs) and small insertions and deletions (Indels) for all samples, and assigned the mutations to clonal or subclonal mutations based on cancer cell fractions (CCFs) and their presence in tumors with multi-region sampling, to reveal the genomic landscape of paired PTs and MTs. On average, medians of 944 and 962.5 mutations were detected in PTs and MTs from METS group, respectively. We next inferred somatic copy number alterations (SCNAs), and MTs were observed to harbor a higher SCNA burden than PTs as measured by the weighted Genome Instability Index (wGII, median=0.48 in MTs, and median=0.38 in PTs, p value =0.001). In addition, PTs with extrahepatic metastasis showed a significantly elevated wGII than those in relapse-free groups (median=0.41 in METS versus medians=0.30 in relapse-free group, $p < 0.01$). By summing the number of metastasis significantly enriched SCNAs in each tumor, we built an ensemble risk score for metastasis. The patients with higher risk score (equal or higher than median metastasis SCNA counts) experienced a significant shorter time to metastasis (TTM) in our discovery cohort ($p < 0.001$) and validation cohort (p=0.009). The phylogeny of all

sequenced tumors within the same patients were reconstructed to trace the clonal origin of MTs, and our results suggested that PT, genetically similar intrahepatic metastasis (IM)-RT, and MT (except for multicentric RT) showed capability to seed MTs, with PT-derived MTs accounting for the majority of cases (85%). We next classified the emergence of metastatic precursors in PTs to explore the timing of metastatic dissemination based on genetic divergence (the proportion of shared mutations among all mutation union) between paired PTs and MTs into three categories: early (0–0.33), intermediate (0.33–0.67), and late (0.67–1.0). Early metastatic dissemination implies significant genetic divergences between PT and MTs. Compared with MT-private mutations, PT-private mutations accounted for most of the genetic divergence. Intra-metastatic heterogeneity was remarkable both in terms of the genetics and immune microenvironment, which resulted from a combination of polyclonal seeding and different strategies to escape immune surveillance. Finally, we found that subclonal Wnt mutations were not selected during spreading. Compared with immune deserted microenvironment of Wnt mutated primary tumors, Wnt wild-type counterparts exhibited reactive immune microenvironment, higher levels of cancer-associated fibroblast infiltration and epithelial-to-mesenchymal transition. **Conclusion:** Our preliminary results reveal complex evolutionary trajectories and furthers our understanding of the molecular modalities of clonal selection during metastatic progression. **Keywords:** hepatocellular carcinoma; metastasis; multicentric tumors; intratumor heterogeneity; cancer evolution.

HNC-13

Unraveling morphological heterogeneity and clinical relevance of circulating tumor cells using an artificial intelligence-based image recognition system

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Aims: Until now, no study has yet systematically unraveled the morphological heterogeneity of CTCs and investigated their clinical indications. This study aimed to establish a Artificial Intelligence (AI)-based system to identify CTC by morphological features and improve the accuracy and efficiency of CTCs analysis. **Methods:** In this study, a total of 2,467 tumor patients of 7 types [Hepatocellular carcinoma, HCC (n = 924), cholangiocarcinoma, CCA (n = 127), breast cancer, BRAC (n = 42), colorectal cancer, CRC (n = 106), gastric cancer, GC (n = 386), lung cancer, LC (n = 68), other cancers (n=58)] treated in Zhongshan hospital, Fudan University were enrolled. All patients accepted CTC tests using ChimeraX®-i120 platform, isolated cells were scanned and digitized by Operetta CLS system (PerkinElmer, USA). Among them, 1,705 patients with 9,692 CTCs in total were included. 57 morphological features of the cells in the digitized images were collected and subjected to an AI-based classification system construction. A two-type cell deep-learning classifier was trained to distinguish CTCs from other cells. Prediction performance was evaluated internally and externally using the areas under the ROC curve (AUC) and the precision-recall curve (PR). To further evaluate the morphological heterogeneity of CTCs across cancers and patients, cells were clustered into different subtypes based on their morphological features. Clinical relevance of CTC morphological subtypes was comprehensively evaluated in a large cohort of patients with HCC. A validation cohort of 77 patients with HCC was further enrolled to validate the clinical significance of CTC morphological subtypes. **Results:** Our AI image-based deep learning CTC identification system can accurately identify CTCs from other blood cells in the fluorescent images, with an AUC more than 0.99 for ROC and an AUC of 0.819 for PR, respectively. We clustered CTCs from 7 cancer types into 6 clusters based on their morphological features. In 6 CTC clusters, CTCs express high morphological variability, cluster 2 and cluster 4 feature in big size of nucleus and cytoplasm among clusters and cluster 6 featured in high intensity. Though CTC count is similar, we found CTCs exhibited great morphological heterogeneity between different cancer types. From the histological subtypes point of view, CTC from adenocarcinoma were more likely clustered in cluster 5&6 while CTCs from HCC were concentrated in cluster 1&2 (P<0.001). Different cancer types had distinct pattern of CTC morphological clusters distributions (Cramér's V = 0.528). We then evaluated the degree of intra-cancer morphological heterogeneity of CTCs. We found that CTCs from HCC and CCA had the highest values of internal heterogeneity while CTCs from GC and BC had the lowest, indicating a similar composition of CTC subgroups. In single patient level, we found 77.6% patients' CTCs only confined to one or two morphological clusters, which indicated the morphological differences largely depend on the origin of cancer. In HCC, we further obtained three CTC morphological subgroups with different features in shape, fluorescence intensity and texture complexion. CTCs with high expression of epithelial cell adhesion molecule, pan-cytokeratin and complex cellular texture was defined as S-III CTC, which was significantly associated with advanced clinicopathologic traits of HCC patients. We found patients who had the occurrence of S-III CTCs were more likely to be suffered from poor prognosis after curative resection (61.3% vs 36.7%, P=0.001) and less effective treatment response during anti-programmed cell death protein 1 (anti-PD-1) therapy (PD:78.3% vs 47.1%, P=0.015). Moreover, we found that CTC morphological subgroups could serve as a more precise biomarker for the dynamic surveillance of tumor progression for HCC patients. **Conclusion:** In this study, we first comprehensively unraveled the morphological heterogeneity and clinical relevance of CTCs using an AI image-based identification and classification system. This deep-learning-based CTC image identification system can accurately identify CTC in an easy, cost-effective, and highly applicable manner. We observed great morphological heterogeneity of CTCs in the levels of cancer types and patients. In HCC, a S-III CTC subgroup were first identified to be associated with poor progression and less effective treatment response for anti-PD-1 therapy. In sum, our system demonstrated great potential to utilize the informative morphological features of CTCs by AI technology, which may provide valuable guidance in clinical management of cancer patients. **Keywords:** deep learning; circulating tumor cell; cell morphology; HCC.

HNC-14

CD36+ cancer-associated fibroblasts provide immunosuppressive microenvironment for hepatocellular carcinoma via secretion of macrophage migration inhibitory factor

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Aims: Hepatocellular carcinoma (HCC) is an immunotherapy-resistant malignancy characterized by high cellular heterogeneity. The diversity of cell types and the interplay between tumor and non-tumor cells remain to be clarified. **Methods:** Herein, we performed single-cell RNA sequencing (scRNA-seq) analysis on unselected viable cells from 11 human HCCs, adjacent samples and 7 CTNNB1N90;Trp53KO murine HCC tumors to elucidate the comprehensive transcriptomic landscape and intercellular communication network. The results of the analyses were validated using multiplex immunofluorescence staining, bulk transcriptomic datasets, and functional in vitro and in vivo experiments. **Results:** Single cell RNA sequencing of human and mouse HCC tumors revealed cancer-associated fibroblast (CAF) heterogeneity. Cross-species analysis determined the prominent CD36+ CAFs exhibited high-level lipid metabolism and expression of macrophage migration inhibitory factor (MIF). Lineage-tracing assays showed CD36+CAFs were derived from hepatic stellate cells. Furthermore, CD36 mediated oxidized LDL uptake-dependent MIF expression via lipid peroxidation/p38/CEBPs axis in CD36+ CAFs, which recruited CD33+ myeloid-derived suppressor cells (MDSCs) in MIF- and CD74-dependent manner. Co-implantation of CD36+ CAFs with HCC cells promotes HCC progression and initiation in vivo. Finally, MIF inhibitor synergizes with anti-PD-1 immunotherapy by restoring antitumor T-cell responses in HCC. **Conclusion:** Our work underscores the importance of elucidating the function of specific CAF subset in understanding the interplay between the tumor microenvironment and immune system. **Keywords:** hepatocellular carcinoma; cancer-associated fibroblasts; myeloid-derived suppressor cells; lipid peroxidation; immunotherapy.

HNC-15

Targeting SRSF1 renders hepatocellular carcinoma eradicable by dual action on tumor and T cells

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Aims: Hepatocellular carcinoma (HCC) is an immunotherapy-resistant malignancy. A number of cancer drugs activate host immune pathways in tumor cells but unfortunately also compromise anti-tumor immune function. It is important to develop tumor cell targeted drugs which enhance rather than compromise immune function. **Methods:** The expressions of SRSF1 in 200 HCC samples and public databases were analyzed. Seahorse XF96 respirometry assays were performed to explore the associations between SRSF1 and glycolytic metabolism in tumor and CD8+ T cells. SRSF1-regulated genes and its binding motif were identified by RNA-seq and RIP-seq. SRSF1-specific small molecule inhibitor were developed to explore potential therapeutic targets in HCC. Additionally, SRSF1-CD8+ T cells conditioned knockout mice were established to explore the effects of SRSF1 on HCC progression in vivo. CD8+ T cells that were co-cultured with tumor cells were sorted by flow cytometry assays. **Results:** We discovered that knockout of SRSF1, an RNA-binding protein, elicited beneficial anti-tumor activity in CD8+ T cells and tumor cells. In CD8 T cells, SRSF1 depletion reshapes metabolic programs and enhances anti-tumor immune function. In tumor cells, SRSF1 inactivation decreased transcription factors c-myc, c-Jun and JunB, which allows the rewiring of glycolytic metabolism. SRSF1 knockdown impairs the glycolytic activity of tumor cells, which restores the function of CD8 T cells, thereby inhibiting tumor growth. These dual antitumor effects by depleting SRSF1 in both tumor and CD8 T cells prompt us to develop a potent small molecule inhibitor of SRSF1, TN2008, elicited effective antitumor immunity and sensitized resistant tumors to checkpoint blockade. **Conclusion:** Targeting SRSF1 thus offers an opportunity to enhance immune function while simultaneously sensitizing resistant tumor cells to immune attack. **Keywords:** resistant tumors; splicing factors; metabolic reprogramming; immunotherapy.

HNC-16

Suppressor of zeste 12 / chemokine receptors 7 axis potentially promotes portal vein invasion in hepatocellular carcinoma

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Aims: Suppressor of zeste 12 (SUZ12), one of the subunits of Polycomb repressive complex 2 (PCR2), is involved in diverse physiopathology processes. This study investigated the potential roles of SUZ12 in hepatocellular carcinoma (HCC). **Methods:** Tissue microarray based on 88 tumor tissues and 27 normal liver tissues was used to examine the expression level of SUZ12. The gain- and loss- of function analysis were conducted to evaluate the effects of SUZ12 on the proliferation, migration and invasion of HCC cells. Meanwhile, luciferase reporter assay and RT-PCR assay were conducted to examine the effect of SUZ12 on transcriptional activity of chemokine receptors 7 (CXCR7). **Results:** The expression level of SUZ12 was positively associated with HCC development, as revealed by tissue microarray analysis. Further gain- and loss- of function analysis demonstrated that SUZ12 promoted the proliferation, migration and invasion of HCC. Mechanistically, we found that SUZ12 could upregulate the expression of CXCR7 at the transcriptional level in HCC cells, and CXCR7 was revealed to contribute to the tumor-promoting roles of SUZ12. Of interest, high SUZ12 expression was positively correlated with CXCR7 in advanced patients with portal vein tumor thrombus. **Conclusion:** Our findings indicate that SUZ12 plays pro-oncogenic roles in the progression of HCC, partially by activating CXCR7 signaling, especially in HCC patients with portal vein tumor thrombus. The SUZ12/CXCR7 axis may serve as a potential therapeutic target for treatments of advanced HCC patients. **Keywords:** suppressor of zeste 12; chemokine receptors 7; hepatocellular carcinoma; portal vein tumor thrombus; invasion.

HNC-17

GOLM1 for predicting survival in patients with hepatocellular carcinoma potentially involved in STAT3 function

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Background: The carcinogenesis and prognosis of hepatocellular carcinoma (HCC) involve complex molecular mechanisms, and Golgi membrane protein 1 (GOLM1) is related to the development and therapeutic efficacy of HCC, but the biological mechanism of GOLM1 in HCC has not been elucidated. **Methods:** In this study, we used The Cancer Genome Atlas (TCGA), GSE14520, GSE62232, and GSE84006 datasets to obtain transcriptome data of HCC patients with corresponding clinical information, and examined the expression and prognosis of GOLM1 in HCC. Additionally, the PPI network of GOLM1 was constructed and candidate genes were identified. Consensus clustering analysis was performed to stratify HCC patients into different subtypes and compared them in OS. Differentially expressed genes (DEGs) between HCC and controls or subtypes were identified. Subsequently, the Cox regression and least absolute shrinkage and selection operator (LASSO) regression analysis were combined to construct the signature genes and the receiver operating characteristic (ROC) curve was used to validate the predictive capability. Then we explored the correlations of the risk signature genes with tumor-infiltrating immune cells and checkpoints. **Results:** In all datasets, there were 2375 common DEGs with the same expression direction, and GOLM1 expression was high in HCC patients with poor prognosis. The PPI network of GOLM1 was mainly involved in STAT related functions, HIF-1 signaling pathway, and PPAR signaling pathway. In addition, molecular docking predicted that GOLM1 had the highest binding energy to STAT3, and IHC further confirmed that the two proteins' expressions were positively associated in HCC patients' tissues. Candidate genes which effected prognosis were identified in network genes intersected with common DEGs. Based on potential genes, the HCC samples were divided into cluster 1 (poor prognosis) and cluster 2 in GSE14520 dataset. Then, we constructed a nomogram model based on signature genes and discovered that the High-risk group had a poorer prognosis than the Low-risk group. Additionally, GOLM1 showed positive correlations with Macrophages, Th2 cells, T helper cells, and checkpoints, whereas negative correlations with eosinophils and Tgd. **Conclusion:** In conclusion, this study demonstrated that GOLM1-related risk signature genes might be employed as a predictive tool in HCC patient survival evaluation and immunotherapy, and GOLM1 may promote HCC through STAT3 and immunosuppression. **Keywords:** hepatocellular carcinoma; GOLM1; STAT3; signature; prognosis; immune.

HNC-18

ESPL1 is elevated in hepatocellular carcinoma and predicts prognosis

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Aims: The extra spindle pole bodies-like 1 (ESPL1) gene is associated with malignant biological behaviors in several tumors. Nevertheless, the correlation between hepatocellular carcinoma (HCC) and ESPL1 has not been determined. The present study analyzed the molecular function and prognostic value of ESPL1 in HCC. **Methods:** Samples from 121 HCCs and 119 adjacent normal tissue specimens were subjected to next-generation sequencing. Clinicopathological and genetic data of HCC patients in The Cancer Genome Atlas (TCGA) were also collected. ESPL1 expression was assessed in 20 pairs of HCC and normal liver specimens by qRT-PCR. The prognostic value of ESPL1 expression was determined by Cox univariate and multivariate regression analyses. ESPL1-related co-expressed genes were evaluated by weighted gene co-expression network analysis (WGCNA). Processes and pathways involving ESPL1 in HCC were determined by Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. The prognostic values of hub genes were determined by joint effect survival analysis. **Results:** RNA-Seq and RT-qPCR showed that ESPL1 expression was significantly higher in HCC than in normal liver tissues ($P < 0.001$). Increased ESPL1 expression (hazard ratio [HR]=2.004, 95% confidence interval [CI]=1.072–3.743, P=0.032), greater tumor size (HR=2.468, 95% CI=1.137–5.354, P=0.021) and advanced BCLC stage (HR=1.956, 95% CI=1.037–3.692, P=0.038) were independently prognostic of poorer overall survival (OS); and increased ESPL1 (HR=1.653, 95% CI=1.032–2.645, P=0.036) and advanced BCLC stage (HR=2.217, 95% CI=1.348–3.646, P=0.002) were independently prognostic of poorer recurrence-free survival (RFS). WGCNA showed that the top 10 co-expressed genes associated with ESPL1 were GTSE1, KIF18B, BUB1B, GINS1, PRC1, KIF23, KIF18A, TOP2A, NEK2 and FANCD2. Enrichment analysis indicated that ESPL1 and its co-expressed genes might be involved in the cell cycle and cell division of HCC. Joint effect survival analysis showed that the mortality rate was approximately 3.37 times higher in HCC patients with high than low expression of ESPL1, GTSE1, BUB1B, PRC1, KIF23, and TOP2A. Tumor infiltration by B cells, dendritic cells, and Treg cells was positively associated with ESPL1. **Conclusion:** ESPL1 might be associated with immunosuppression and might be an effective prognostic indicator in patients with HCC. **Keywords:** hepatocellular carcinoma; extra spindle pole bodies-like 1; prognosis.

HNC-19

ISG15-induced mitochondrial remodeling promotes CD8+T cell memory differentiation that contributes to clinical ICIs treatment

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Background/Aims: Though Immune related adverse events (irAEs) impede the application of immune checkpoint inhibitors (ICIs), growing number of clinical trials have revealed a positive correlation between the irAEs onset and cancer patients' better prognosis. However, the explanation for this phenomenon is still lacking, uncovering the underneath mechanisms may provide targets for optimizing cancer immunotherapy. **Methods:** We desingned a retrospective case-control study (registration number: ChiCTR2200061527) and found abnormal cytokine levels in the irAE population; We used flow cytometry analysis and single-cell sequencing analysis to exam clinical samples; Primary CD8+ T cell models were constructed using mouse spleens or human PBMCs (from healthy donors) and a series of ex vivo experiments were designed to detect changes in genes transcription, protein translation, cell differentiation and oxidative respiration levels. We further confirm our finding by construction of conditional KO mice and ACT model. **Results:** we established clinical and single cell transcriptomics profiles of the irAE entity and analyzed the prognosis-related factors. We revealed an ISG15+CD8+T cell subset characterized by high expression of ISGs and ISGylation in irAE patients, whom we found to possess higher memory T cell (Tmem) proportion. Further molecular and functional studies showed the elevation of ISG15/ISGylation promote mitochondrial fusion through modulating mitochondria inner membrane complex, especially Optic Atrophy 1(OPA1), who gained enhanced protein stability within its ISGylated form. Loss of ISG15/ISGylation in CD8+T cells cause reduced cytotoxicity, impaired fatty acid oxidase (FAO) metabolism, and impeded mitochondria fusion as well as Tmem differentiation. **Conclusion:** Our study explicated the essential role of ISG15/ISGylation in mitochondrial inner-membrane modulation and Tmem cell generation. It partially attributes to the irAE entity's better prognosis and be a potential target to fuel cancer immunotherapy. **Keywords:** ISG15; mitochondrial remodeling; T cell.

HNC-20

High expression of STK32C is an independent predictor of poor prognosis in HCC

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Aims: Hepatocellular carcinoma (HCC) is the main type of liver cancer. STK32C was found to be high-expression in brain tissue. However, the role of STK32C in HCC still lacks research. We aimed at investigating the diagnostic and prognostic value of STK32C in HCC. **Methods:** TCGA, GTEx, HCCDB, TISIDB, TIMER, and HPA were conducted for bioinformatics analysis. The expression of STK32C in HCC was verified by immunohistochemistry. Meanwhile, Western blot, qRT-PCR and other phenotypic experiments were performed for validation. **Results:** Bioinformatics analysis showed that the expression of STK32C was upregulated in HCC, which was verified in IHC. In addition, the proliferation, migration and invasion of HCC cells were inhibited by knocking down STK32C. Meanwhile, the transcript levels of STK32C showed significant correlation with age, weight, gender, histological grade of HCC patients. Survival analysis indicated that STK32C had significant clinical prognostic value in HCC. ROC and nomogram demonstrated the strong predictive capacity of STK32C. Meanwhile, we found that STK32C was correlated with neuroactive ligand-receptor interactions and c-AMP signaling pathway in enrichment analysis. Immune infiltration analysis indicated that STK32C was associated with tumor purity, macrophage, neutrophil, B cells, etc. **Conclusion:** We found the over-expression of STK32C promotes the progression of HCC and STK32C could be used as an independent predictive marker for HCC. Our research provides new target for HCC therapy. **Keywords:** HCC; STK32C; progression; diagnosis; immune.

HNC-21

Uncovering the heterogeneity and clinical relevance of circulating tumor-initiating cells in hepatocellular carcinoma using an integrated immunomagnetic-microfluidic platform

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Aims: Circulating tumor-initiating cells (CTICs) are specifically referred to these CTCs subpopulation with stem cell-like properties, playing pivotal roles in tumor metastasis and recurrence. However, little is known about the biology and clinical relevance of CTICs in hepatocellular carcinoma (HCC). Here, we investigated the molecular heterogeneity and clinical relevance of CTICs in HCC using a novel integrated immunomagnetic-microfluidic platform (iMAC). **Methods:** The two-layer microfluidic chip was fabricated by Micro-Electro-Mechanical System technology. A four-channel microfluidic chip was applied to investigate the composition of CTICs in patients with primary and recurrent HCC utilizing microbeads labeled with one of four stem-related markers: epithelial cell adhesion molecule (EpCAM), CD133, CD90 and CD24. In addition, the CTICs detection in benign liver diseases patients was performed. Using samples from the 33 HCC patients, we also performed a paired comparison to determine the capabilities of the iMAC vs. the CellSearch system. The dynamic changes of these four CTICs subsets were serially monitored during treatment courses. Finally, the eluted CTICs were collected for downstream cytological and molecular analysis, in which single-cell RNA profiling was used to reveal the molecular characteristics of the four CTICs subsets. **Results:** The data suggest that iMAC platform could isolate CTICs with high throughput (2 mL/hour), high efficiency (>95% recovery rate with a 4 mL blood sample), and dual functional modes (retaining or retrieving mode). The iMAC platform detected significantly more EpCAM+ CTICs in samples from 33 HCC patients than the FDA-approved CellSearch system detected from the same samples (0.92 ± 0.94 vs. 0.23 ± 0.36, $P < 0.001$). EpCAM+ CTICs counts were significantly higher in recurrent patients than the nonrecurrent.

Receiver-operating characteristics curve analysis indicated that a cutoff value of ≥0.75 CTICs/mL blood was the most significant in predicting postoperative recurrence. Patients with higher levels of CTICs (≥0.75/mL) had significantly shorter time to recurrence (median, 9.6 months vs. not reached) and higher recurrence rates (73.5% vs. 20.5%) than those with lower levels of CTICs. Furthermore, the distinct stem-related markers expression of CTICs could distinguish primary HCC, recurrent HCC and transcatheter arterial chemoembolization (TACE)-resistant HCC. In newly diagnosed HCC, 85.71% (12 of 14) patients with detectable CTICs had EpCAM+ CTICs, and the majority of isolated CTICs were EpCAM+ cells. By contrast, CTICs detected from recurrent HCC patients were mostly CD90+ cells. In addition, the dynamic shifts in CTICs subsets were associated with the response to TACE treatment. For patients with TACE-resistant tumors, the predominant proportion of CTICs switched to CD24+ and CD90+. For patients with tumors that responded to TACE therapy, few CD24+ CTICs could be detected before or after TACE. By contrast, serum alpha fetoprotein (AFP) levels were not able to reflect the response to TACE or tumor relapse in time. Single-cell transcriptional profiling further revealed marked heterogeneity among individual CTICs and separated the four CTICs subsets into distinct phenotypes. Each CTIC expressed at least one putative liver TICs biomarker (EpCAM, CD133, CD90, CD24, or ICAM-1). CTICs expressed a diverse set of stem cell-related genes and key genes that are active in several cancer stem cell signaling pathways. Moreover, most CTICs were dual positive for epithelial, as well as mesenchymal markers. The anti-apoptotic genes were highly enriched in CTICs. In contrast, pro-apoptotic genes were negative for all cells profiled. **Conclusion:** Using an integrated immunomagnetic-microfluidic platform, this study identified great heterogeneity of CTICs composition and molecular characteristics at the single-cell level, which were closely associated with different disease states and therapeutic response. Dissecting the heterogeneity of CTICs using the iMAC platform represents a novel and informative method for accurate CTICs characterization. This innovative technology may enable more in-depth cancer biology research and clinical cancer management than is currently available. Changes in CTICs numbers closely correlated with HCC tumor burden. **Keywords:** circulating tumor-initiating cells; hepatocellular carcinoma; immunomagnetic-microfluidic platform; heterogeneity.

HNC-22

Hypermethylation-mediated transcriptional silencing of lncRNA-SCARF1 promotes progression and metastasis of hepatocellular carcinoma

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Background/Aims: Hepatocellular carcinoma (HCC) is one of the most common causes of cancer-related deaths. Recent studies have demonstrated that deregulation of long noncoding RNAs (lncRNAs), such as abnormal DNA methylation of promoter, is strongly associated with development and progression of diverse malignant tumors. This study investigated the mechanisms and changes in DNA methylation levels of promoter regions of HCC-specific lncRNAs, and alterations of downstream target genes. **Methods:** LncRNA expression profile data of 8 human HCC tissues and matched normal tissues were obtained. LncRNAs with aberrant methylation were identified through DNA methylation microarray. The biological functions of the lncRNAs were investigated through targeted knockdown of lncRNA-SCARF1 in vitro and in vivo. Furthermore, the downstream targets of lncRNA-SCARF1 were identified through ChIRP-MS. **Results:** LncRNA-SCARF1 was significantly down-regulated in HCC samples. Hypermethylation in the promoter of lnc-SCARF1 induced its down-regulation in HCC. Over-expression of lnc-SCARF1 inhibited the tumor proliferation and migration ability of HCC cells in vitro and in vivo. Furthermore, CUL9 was found to be a potential downstream target of lncRNA-SCARF1. **Conclusion:** LncRNA-SCARF1 regulates HCC progression by interacting with CUL9 and may serve as a prognostic biomarker or an effective therapeutic target in HCC. **Keywords:** hepatocellular carcinoma; LncRNA; Microarray; CUL9.

HNC-23

CircESYT2 induces lenvatinib resistance in hepatocellular carcinoma via up-regulating KSR1 by sponging miR-331-3p

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Aims: Lenvatinib was recommended as the first-line therapy for patients with advanced-stage hepatocellular carcinoma (HCC). However, the inherence and development of drug resistance is still a major obstacle to improve clinical outcomes. Recently, the dysregulation of circular RNA (circRNA) has been proven to exert critical roles in the drug resistance of cancers, including HCC. This study aimed to reveal the key circRNA and its mechanism in inducing Lenvatinib resistance to HCC. **Methods:** Under Lenvatinib treatment with gradually increasing the concentration, both HepG2 cell line and MHCC97-H cell line resisting to Lenvatinib (HepG2-R and MHCC97H-R) were obtained. The differentiated circRNAs between the drug-resistant cells and the parent cells were detected by circRNA-seq. The most elevated circRNA were selected for subsequent studies. The expression of the selected circRNA in HCC cell lines and tissues were detected by qRT-PCR and fluorescence in situ hybridization (FISH) respectively. Then, roles of the selected circRNA in Lenvatinib resistance were determined by regulating this circRNA expression (over expressed and knocked down). The potential miRNAs sponged by the selected circRNA were identified via the prediction and dual-luciferase reporter assay. Moreover, the interactions between the identified miRNA and mRNA of KSR1 were further verified by RNA immunoprecipitation (RIP) and RNA interference. **Results:** We found that circESYT2 was the most up-regulation in the drug-resistant cells compared to the parent cells. Furthermore, the circESYT2 was discovered to be up-regulated in HCC compared to the peritumor tissues. Compared to those who benefited from Lenvatinib, the expression of circESYT2 in tumor from patients who resisted to Lenvatinib was significantly high. Importantly, HCC patients with higher circESYT2 had lower overall survival rate than the patients with lower circESYT2. The increased expression of circESYT2 in

HCC cells promotes tumor proliferation, migration and invasion. HepG2 overexpressing circESYT2 showed more migrated, invasive and less apoptotic, the subcutaneous tumors were lager compared to vector group under Lenvatinib treatment. Mechanistically, circESYT2 could sponge miR-331-3p to increase the expression of the KSR1 resulting in activating RAS/RAF/ERK pathway, which could counteract the effect of Lenvatinib. **Conclusion:** these results uncover the effects of circESYT2 on HCC progression and Lenvatinib resistance, and provide a potential target for overcoming the resistance to Lenvatinib. **Keywords:** CircRNA; hepatocellular carcinoma; lenvatinib.

HNC-24

Histological features and local immune microenvironment change during liver regeneration in associating liver partition and portal vein ligation for staged hepatectomy (ALPPS)

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Aims: To compare histologic features and local immune microenvironment of liver regeneration in first stage and second stage of patients who received ALPPS. **Methods:** Liver tissues obtained from 22 patients underwent ALPPS were used for hematoxylin and eosin (H&E) staining, immunohistochemistry (IHC) staining of immune cell clusters and electron microscopy. Three different ALPPS rat models in different sampling time after ALPPS (0d, 1d, 2d, 4d, and 7d) were constructed for the validation of findings from human liver samples. **Results:** After an interval of 7~28 days (mean: 15.0 days) between stage I and stage II, FLR of patients was observed to increase significantly by 42.3% (P< 0.001), totally relative hepatocyte number increased by 48.0% (P< 0.001). From electron microscopy images of liver tissues, increased endoplasmic reticulum (ER) and increased extracellular matrix (ECM) were observed in stage I compared with stage II of ALPPS. Transient dysfunction of liver function were observed from postoperative day (POD) 1 to POD 3 with significant increase in sTBL, ALT, AST from POD 1 and gradual drop from POD 3 to POD 7. After stage I of ALPPS, consistent with the trend of the number of white blood cells, neutrophils in the whole blood were observed to elevate significantly from $(3.50\pm1.17)\times10^9$/L in Pre 1 to $(9.70\pm3.52)\times10^9$/L in POD 1 and gradually decreased from POD 3 to POD 7. While, the cell number per HPF (40×) dropped in postoperative 1~4 days and rebounded afterwards. Lagging behind proliferative hepatocytes, Ki-67 positive cholangiocytes/all cholangiocytes increased dramatically to the peak in POD 2, and then dropped significantly almost close to the sham in POD 4. Consistent with human sample findings, MPO staining of FLR of three ALPPS rat models began to increase significantly from POD 2~ POD 4 compared with POD 0. **Conclusion:** During stage II to stage I of ALPPS, FLR volume increased significantly with proliferation of hepatocytes, cholangiocytes, and hypertrophy of hepatocytes as well as increased ER and increased ECM. Proliferation peak of cholangiocytes dropped behind hepatocytes in proliferative phase after the Stage I of ALPPS. Significantly infiltration of neutrophils was observed

	Stage 1	Stage 2	P-value
Future liver remnant (FLR)	400.3 ml	569.2 ml	P < 0.001
Ki-67 positive hepatocytes percentage	0.39%	2.78%	P < 0.01
Binuclear hepatocyte percentage	5.14%	6.83%	P < 0.001
Bile duct number per portal triad	7.3	10.0	P < 0.05
Hepatocyte-hepatocyte distance	16.3 μm	18.9 μm	P < 0.001
Hepatocyte number per HPF (40×)	608.8	587.6	P = 0.40
MPO positive cells (neutrophils mainly) per 20×HPF	84.95±64.56	181.5±197.2	P < 0.001
FLR/ weight ratio			
Zurich ALPPS	0.46	1.00	P < 0.001
30% ALPPS	0.86	2.07	P < 0.001
10% ALPPS	0.46	1.29	P < 0.001
Ki-67 positive hepatocytes/all hepatocytes percentage	POD 0	POD 1	POD 2
30% ALPPS model	9.1%	22.2%*	51.3%#
Zurich ALPPS model	9.1%	27.8%*	61.9%#
10% ALPPS model	9.1%	22.7%*	57.5%#
Ki-67 positive cholangiocytes/all cholangiocytes	POD 0	POD 2	POD 4
30% ALPPS model	2.1%	32.5%#	
Zurich ALPPS model	2.1%	26.4%#	
10% ALPPS model	2.1%	37.5%#	

in early stage after stage I of ALPPS based on human and rat ALPPS findings, which might contribute to liver regeneration. **Keywords:** ALPPS; liver regeneration; histologial feature; immune microenvironment.

HNC-25

Autophagy inhibition of macrophages promotes IL-1β-mediated hepatocellular carcinoma progression through inflammasomes activation and self-recruitment

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Aims: To explore the role and function of macrophage autophagy in HCC progression, search the key cytokines contributing to the HCC metastasis and invasion, and elucidate the mechanism by which these cytokines promote HCC progression. **Methods:** The autophagy state of macrophages was detected by the multiplex immunofluorescence histochemistry through identifying the colocalization of CD68 and LC3 and the transmission electron microscope through determining the number of autophagosomes. Kaplan-Meier method was used to evaluate OS and PFS between different groups on our HCC tissue microarray. The targeted protein expression level was examined by western blotting and immunofluorescence, while the targeted mRNA expression was identified by qRT-PCR. Cellular viability was detected using a cell counting kit-8 and the ability of cell invasion and migration was examined by transwell assays. Human cytokine array and enzyme-linked immunosorbent assay were used to confirm the cytokines responsible for HCC metastasis. Orthotopic transplantation of the tumor and tail vein injection of the lung metastasis model were used to determine the efficacy of IL-1β blockade in inhibiting HCC metastasis. **Results:** The macrophages in tumor tissue have lower level of autophagy compared with those in para-tumor tissue, which was associated with poor prognosis and more microvascular invasion in HCC patients. Furthermore, we found that HCC cells can suppress the macrophage autophagy initiation through the upregulation of mTOR and ULK1 phosphorylation level at Ser2448 and Ser757, respectively. Mechanically, autophagy-mediated macrophage IL-1β production exhibited a direct metastasis-promoting effect via autophagy-NLRP3-ASC-IL-1β axis. In addition to direct metastasis-promoting effect, decreased macrophages autophagy facilitated the HCC progression through inducing macrophage infiltration via CCL20-CCR6 axis so that recruited macrophages can secrete more IL-1β and CCL20 to promote HCC metastasis and remodel tumor microenvironment. Through the lung metastasis model in vivo, we provide evidence that targeting IL-1β by mAb or using IL1R1 antagonist Raleukin impairs IL-1β-promoted HCC metastasis in vivo. **Conclusion:** Our results elucidate the state of autophagy suppression of tumor macrophages, which builds a positive regulatory loop between HCC cells and macrophages through the secretion of IL-1β and CCL20 to remodel HCC microenvironment and promote HCC progression. We provide evidence on supporting the interruption of this metastasis-promoting loop through IL-1β blockade may provide a promising therapy strategy for HCC patients. **Keywords:** macrophages; autophagy; tumor microenvironment; hepatocellular carcinoma; inflammasome.

HNC-26

The mechanism and intervention of immune metabolite L-2-HG in inhibiting T cell exhaustion

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Aims: The molecular mechanism of CD8+ T cell exhaustion is still unclear. This study intends to investigate whether the metabolic intermediate L-2-HG is related to T cell exhaustion, and the mechanism of L-2-HG in regulating T cell exhaustion through epigenetics. **Method:** We selected three conditions (HIV infection, chronic leukemia, HCC) to detect peripheral blood or tumor tissue of patients. Metabolism and L-2-HG and D-2-HG content in PD-1+ T cells and PD-1- T cells were checked. Epigenetic signatures and transcriptome alterations in exhausted T cells were described by combined RNA-seq and ATACseq analysis. In addition, an in vitro exhaustion model was established by chronic repeated anti-CD3/CD28 stimulation, and flow cytometry was used to detect the mitochondrial mass, membrane potential, ROS, mPTP opening status, etc. The effects of L-2-HG levels on mitochondrial function, differentiation, and anti-tumor effects of exhausted CD8+ T cells were evaluated by in vitro cell culture and animal tumor models. **Results:** We found mitochondrial depolarization and metabolic dysfunction in exhausted CD8+ T cells, the metabolic intermediate 2-HG was significantly reduced, and L-2-HG was the main change. In addition, the results of RNA-seq and ATACseq analysis showed that the epigenetic characteristics of exhausted CD8+ T cells were altered, including significantly increased levels of H3K27me3, which blocked cell differentiation. L-2-HG treatment of exhausted T cells showed that the mitochondrial metabolism of T cells improved , with decreased H3K27me3 and enhanced memory T cell differentiation. A mouse melanoma tumor model was established, and the activated CD8+ T cells treated with L-2-HG were given adoptive therapy. It was found that the tumor volume was reduced in the treatment group, and the effector function of T cells was enhanced. **Conclusion:** The study found impaired mitochondrial polarization, metabolic dysfunction, and reduced L-2-HG in exhausted CD8+ T cells. L-2-HG was treated in exhausted CD8+ T cells, mitochondrial metabolism, and epigenetic changes. Therefore, we hypothesized that L-2-HG acted as an immune metabolite through epigenetic modifications, reversed the progression of exhausted T cells and improved exhausted T cell effector function and antitumor immunity. **Keywords:** immunometabolism; CD8+ T cells; T cell exhaustion; H3K27me3; L-2-HG.

Cholangiocarcinoma-clinical

CC-01

Molecular features and clinical implications of perineural invasion in intrahepatic cholangiocarcinoma

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Aims: Perineural invasion (PNI) is an important mechanism associated with metastasis in malignancies, including intrahepatic cholangiocarcinoma (ICC), and is correlated with poor prognosis. However, the molecular features and their relationship with the prognosis of ICC patients with PNI remain unclear. Our study aims to reveal the molecular characteristics and clinical significance of PNI in ICC. **Methods:** Prognostic implications of PNI were investigated in MSK cohort and was validated in our independent cohort of patients with ICC using the Kaplan–Meier method. PNI-related genomic profiles were analyzed in the MSK cohort, and the PNI-related transcriptomic atlas was examined in the ZS-ICC cohort from our team. GO, KEGG, and ssGSEA analysis were performed. Immunohistochemistry was used to investigate the relationship between PNI and markers of neurons, hydrolases, and immune cells. The efficacy of adjuvant therapy in ICC patients with PNI was also assessed. **Results:** A total of 30.6% and 20.7% patients with ICC had PNI in the discovery and validation cohorts respectively. Patients with PNI in both cohorts presented with shortened overall survival (OS) and relapse-free survival (RFS). The PNI showed positive expression of tyrosine hydroxylase (TH), a marker of sympathetic nerves. Patients with PNI showed a high frequency of KRAS mutations, decreased NK cell infiltration, increased neutrophil infiltration, and elevated expression of PD-L1, CD80, and CD86. Patients with PNI benefited from an extended OS after adjuvant therapy with TEGIO, GEMOX, or capecitabine. **Conclusion:** Altogether, our study deciphered the molecular features of a high frequency of KRAS mutation, positive TH expression, increased neutrophil infiltration, and decreased NK cell infiltration in ICC patients with PNI. Patients with PNI showed a poor prognosis after surgery but a good response to adjuvant chemotherapy. **Keywords:** perineural invasion; intrahepatic cholangiocarcinoma; sympathetic nerve; KRAS mutation; immune infiltration.

CC-02

T cell immunoglobulin and ITIM domain (TIGIT) and programmed death ligand-1 (PD-L1) act together to maintain an inhibitory tumor microenvironment in intrahepatic cholangiocarcinoma

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Aims: T cell immunoglobulin and ITIM domain (TIGIT) plays an important role in sustaining the inhibitory tumor microenvironment. Targeting of TIGIT alone or of TIGIT and programmed death protein 1 (PD1) in combination have yielded promising results in several malignancies. However, in patients with intrahepatic cholangiocarcinoma (ICC), the expression and clinical significance of TIGIT, and its interaction with programmed death ligand-1 (PD-L1), remain unknown. **Methods:** We investigated the expression of TIGIT and PD-L1 using immunohistochemistry staining in 297 patients with ICC. We analyzed the prognostic implication of TIGIT and PD-L1 expression using the Kaplan-Meier method and investigated the interaction between TIGIT and PD-L1 in ICCs. **Results:** We detected TIGIT expression predominantly in tumor-infiltrating lymphocytes (TILs) of ICCs. The number of TIGIT-positive cells in ICC tumor tissues was significantly higher than in corresponding para-tumor tissues (P<0.001). TIGIT expression in tumor samples positively correlated with malignant clinicopathological characteristics. Survival analysis showed that high TIGIT expression was related to poor overall survival (OS) and a high recurrence rate of ICC in patients after operation. Based on different expression levels of PD-L1 in tumors and TIGIT in TILs, Cox regression analysis showed that TumorPD-L1/TILsTIGIT was an independent risk factor of increased recurrence and decreased OS, and patients with high TumorPD-L1/TILsTIGIT showed the poorest prognosis. **Conclusion:** TIGIT is overexpressed in the tumor tissue of ICCs and may be a potential target for immune checkpoint blockade. TILsTIGIT interact with TumorPD-L1 in maintaining the inhibitory immune tumor microenvironment, making combined therapy of anti-TIGIT/PD-L1 for ICC a promising possibility. **Keywords:** intrahepatic cholangiocarcinoma; T cell immunoglobulin and ITIM domain; Programmed death ligand 1; Prognosis.

CC-03

Prognostic factors of 46 cases Liver transplantation for treating intrahepatic cholangiocarcinoma

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Aims: To summarize the therapeutic efficacy of liver transplantation in patients with intrahepatic cholangiocarcinoma (iCCA) and analyze the prognostic risk factors. **Methods:** The clinicopathological data of 46 patients pathologically diagnosed with iCCA who underwent liver transplantation in Zhongshan Hospital Affiliated to Fudan University from April 2001 to March 2022 were analyzed retrospectively. The survival and recurrence of the patients were followed up. Kaplan Meier method was employed to analyze the overall survival (OS) rate and relapse free survival (RFS) rate of patients, and Cox regression model was used to evaluate the risk factors affecting the prognosis. **Results:** The median overall survival time of patients with iCCA after liver transplantation was 19 months, and the 1, 3, 5-year OS rates were 64.4%, 30.2% and 20.7%, respectively. The median RFS time was 10 months, and the 1, 3 and 5-year RFS rates were 45.8%, 20.8% and 10.4%, respectively. The results of multivariate analysis revealed that the level of carbohydrate antigen (CA) 19-9 (P=0.026) was an independent risk factor for the overall survival time of patients, and the extrahepatic invasion of iCCA (p=0.019) was an independent risk factor for tumor recurrence and metastasis. **Conclusion:** The prognosis of liver transplantation for intrahepatic cholangiocarcinoma is poor. The high level of CA19-9 is an independent risk factor for

short postoperative survival of recipients, and direct tumor invasion of extrahepatic tissues is an independent risk factor for high recurrence rate after surgery. **Keywords:** intrahepatic cholangiocarcinoma; liver transplantation; therapeutic efficacy; prognostic factors; 46 cases.

CC-04

The efficacy and safety of durvalumab-based systemic therapy as non-first-line therapy in patients with refractory biliary tract cancer

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Aims: To evaluate the efficacy and safety of durvalumab-based systemic therapy as non-first-line therapy in patients with PD-1 inhibitor resistance biliary tract cancer. **Methods:** This is a single-arm study for a preliminary assessment of the safety and efficacy of the durvalumab-based systemic therapy in patients who were advanced biliary tract cancer without the chance of operation and failed after PD-1 inhibitors treatment. Patients received durvalumab 1500 mg i.v. every 3 weeks. The primary endpoints were objective response rate (ORR) and disease control rate (DCR), which were assessed in the efficacy population. The tumor response was evaluated according to RECIST v1.1 by investigators. The secondary endpoints were overall survival (OS), progression-free survival (PFS), and treatment-related adverse events (TRAEs). **Results:** At the cutoff (June 2022), a total of 14 eligible patients were enrolled, including 10(71.4%) intrahepatic cholangiocarcinoma, 2(14.3%) extrahepatic cholangiocarcinoma and 2(14.3%) gallbladder cancer. All patients failed the previous PD-1 inhibitor-based therapy. As for the treatment regimen, two were treated with durvalumab plus GEMOX, ten were treated with durvalumab plus lenvatinib and two patients were treated with durvalumab combined with lenvatinib plus GEMOX. The median follow-up was 7.76 months. The confirmed ORR in 12 evaluable patients was 25% and the DCR was 66.7%. 3 PR, 5 SD and 4 PD were achieved. The median PFS and OS were not reached. The 6-month PFS rate was 34.63%, and the 6-month OS rate was 73.5%. TRAEs (all grades) occurred in all patients, and 11 patients (78.5%) experienced grade 3 AEs. No grade 4 and fatal AE was observed. The most common TRAEs (any grade) were hypertension (64.29%), ALT or AST elevation (57.1%), fatigue (42.9%), pain (35.7%) and decreased platelet count (35.7%). Regarding the grade 3 TRAEs, the most common AEs were hypertension (28.6%), palmar-plantar syndrome (21.43%), fatigue (14.29%), and pain (14.29%). **Conclusion:** Durvalumab-based systemic therapy as non-first-line therapy has shown a promising benefit in patients with PD-1 inhibitor resistance BTC. A prospective clinical trial with a control arm will validate the present outcomes and explore the potential biomarker. **Keywords:** biliary tract cancer; PD-1; durvalumab.

CC-05

Sequential Radiotherapy and chemotherapy combined with anti-PD-L1 immunotherapy for locally advanced perihilar cholangiocarcinoma: A case report

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Aims: To report a patient with locally advanced pCCA recepted sequential radiotherapy and chemotherapy combined with anti-PD-L1 immunotherapy, the tumors were no longer evident 10 months. **Methods:** Clinical observation. **Results:** A case of 67-years-old patient with locally advanced pCCA, sequential radiotherapy and chemotherapy combined with immunotherapy for 10 month PFS and maintained a good quality of life. **Conclusion:** A case of 67-years-old patient with locally advanced pCCA, sequential radiotherapy and chemotherapy combined with immunotherapy for 10 month PFS and maintained a good quality of life. **Keywords:** locally advanced pCCA; radiotherapy; chemotherapy; anti-PD-L1 immunotherapy; sequential.

Cholangiocarcinoma-non-clinical

CNC-01

Therapeutic effects of Tn-MUC1 chimeric antigen receptor T cells combined with glycolysis inhibitors on intrahepatic cholangiocarcinoma

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Aims: Intrahepatic cholangiocarcinoma (ICC) is a type of liver cancer with high malignancy. Therefore, the search for new treatment methods has always been the urgent focus and difficulty of ICC. CAR-T cell immunotherapy is one of the frontiers of cancer therapy. It is one of the hot spots in solid tumor immunotherapy to explore how to effectively apply this technology to ICC treatment. **Methods:** In this study, we screened specific targets of ICC by immunohistochemistry in a large sample of cases, and then prepared car-T engineered cells of this target to conduct effective function studies in vitro and in vivo, and analyzed their clinical therapeutic values for ICC patients. In addition, we combined with ENOblock, to explore the influence of tumor glucose metabolism on CAR-T cell therapy and the value of drug combination, clarify the relevant mechanism, help to better formulate immunotherapy regimen, optimize CAR-T cell therapy, and obtain the optimal benefits of immunotherapy. **Results:** Among the tumor tissue microarray of 328 ICC patients, Tn-MUC1 was highly expressed in 70.73% cancer tissues, while the corresponding adjacent tissues

were nearly undetected. Survival analysis showed that patients with high expression of Tn-MUC1 had significantly lower 1-year, 3-year, and 5-year survival rates (62.3% vs 75.0%, 36.5% vs 57.0%, 28.5% vs 44.4%) and higher cumulative recurrence rates at 1, 3, and 5 years (52.7% vs 42.3%, 74.7% vs 57.5%, 79.0% vs 65.3%) than patients with low expression of Tn-MUC1.

Then, Tn-MUC1 was used to prepare specific 5E5 CAR-T cells. HUCCT1-KO cells could specifically activate 5E5 CAR-T cells, releasing TNF-α and IFN-γ. Moreover, CAR-T cells could effectively lysate and kill Hucct1-KO cells, while the control Hucct1-WT could not be killed. Finally, in combination with ENOblock, it was found that the cytotoxicity of CAR-T cells on ICC could be affected by glucose metabolism, and the cytotoxicity potential of 5E5 CAR-T cells to Hucct1-KO cells could be effectively increased by interfering with tumor glucose metabolism. **Conclusion:** We identified and verified a new specific target (Tn-MUC1) for ICC, preliminarly completed the construction of related 5E5 CAR-T cells and preclinical studies, and verified the effect of glucose metabolism intervention on T cell therapy. **Keywords:** ICC; T cell; Tn-MUC1; glucose metabolism; glycolysis inhibitor.

CNC-02

Circular RNA MALAT1 promotes intrahepatic cholangiocarcinoma progression by recruiting hepatic stellate cells and regulatory T cells via CCL28 upregulation

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Aims: Intrahepatic cholangiocarcinoma (ICC) is the second most common malignancy arising from the liver. Accumulating evidence has suggested that circular RNAs (circRNAs) are extensively involved in tumor initiation and progression, recent studies focused mainly on their regulatory role in tumor cells per se, but their involvement in the crosstalk between the microenvironment and tumor cells is far from determined. Here, we aimed to identify circRNAs with potential roles in regulating the ICC microenvironment and clarify the underlying mechanisms. **Methods:** CircRNA microarray was performed to screen significantly upregulated circRNAs in hepatic stellate cells(HSCs) co-cultured ICC cells. CCK-8, colony formation, transwell chamber, and mouse xenograft models were used to examine the role of circRNAs in ICC proliferation and metastasis. Immunostaining, flow cytometry, and migration assays were performed to analyze the chemotactic response of hepatic stellate cells and regulatory T cells. Kaplan–Meier plots and Cox regression model were used to elucidate the clinical importance of the identified molecules. **Results:** CircMALAT1(hsa_circ_0002082) was highly expressed in HSCs co-cultured ICC cells, and high expression of circMALAT1 was associated with a worse prognosis following ICC resection. Silencing of circMALAT1 repressed the proliferation, migration, and invasion of ICC cells in vitro and inhibited the growth and metastasis of ICC mouse xenograft in vivo. Furthermore, we found that circMALAT1 could promote HSCs and Tregs infiltration to generate an immunosuppressive tumor microenvironment to promote tumor growth, and this was a CCL28-dependent mechanism. **Conclusion:** Our research demonstrated that circMALAT1 promotes ICC progression via Tumor Cell-Intrinsic and -Extrinsic mechanisms, and circMALAT1 may be a potential target in treating ICC. **Keywords:** intrahepatic cholangiocarcinoma; circular RNA; hepatic stellate cells; regulatory T cells; CCL28.

CNC-03

Tracing the origins of cancer associated fibroblasts in ICC

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Aims: Cancer-associated fibroblasts (CAFs) are heterogeneous and play important roles in modulating intrahepatic cholangiocarcinoma (ICC) progression. The heterogeneity of CAFs are thought to be derived from different cellular origins, including hepatic stellate cells(HSCs) and portal fibroblasts(PFs), but formal fate-tracing studies are lacking. Here, we aimed to identify the origins and contribution of CAFs in ICC. **Methods:** By single-cell RNA sequencing of nonparenchymal cells from mouse liver, we identified Tcf21 as a unique marker that restricted its expression to quiescent HSCs, and CD34 as a marker for portal fibroblasts. To elucidate CAF origins, we used an ICC mouse model by hepatic overexpression of oncogenic driver myr-AKT in combination with NICD1 (NICD/AKT) via the Sleeping Beauty system, delivered by hydrodynamic tail vein injection. **Results:** Tracing Tcf21+ cells by Tcf21-CreER (Cre-Estrogen Receptor fusion protein under the control of Tcf21 gene promoter) targeted ~10% of all HSCs, most of which were located at periportal and pericentral zones. While CD34-CreER was expressed by fibroblasts around the portal veins and bile ducts.After ICC formation, we found Tcf21+ HSC cells generated most CAFs, while CD34-CreER+ cells generated a small population of all CAFs in ICC. Conditional depletion of Tcf21-CreER+cells, but not CD34-CreER+ cells, inhibited liver tumor progression. **Conclusion:** Tcf21-CreER–targeted perivenous stellate cells are the main source of CAFs in ICC. Preventing the expansion/differentiation of Tcf21-lineage CAFs could be effective therapeutic approaches for ICC. **Keywords:** hepatic stellate cells; portal fibroblasts; TCf21; CD34.

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