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From the Editor's desk. . .

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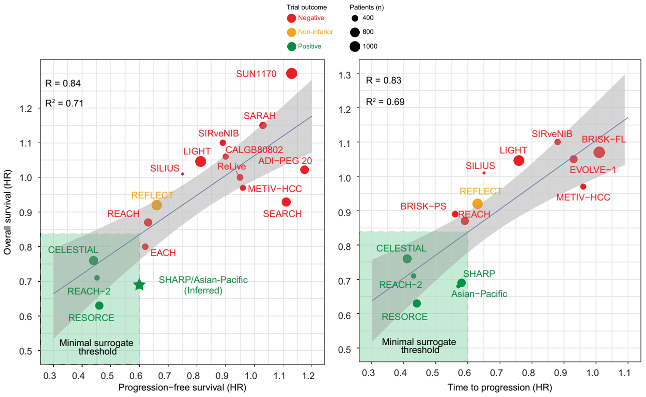
SELECTION OF THE MONTH

Progression-free survival: endpoint for HCC trials?

Around half of patients with hepatocellular carcinoma (HCC) will receive systemic therapies during their life span. A decade of negative drug trials in HCC followed the approval of sorafenib in 2007. However, in the last 2 years several first- and second-line compounds have shown clinical benefit. Thus, there is now a clear need to define surrogate endpoints that both reliably recapitulate survival benefits and can be assessed before additional efficacious drugs are administered. In this issue, Llovet *et al.* conduct a thorough analysis of 21 phase III trials published in advanced HCC, demonstrating a moderate correlation between progression-free survival (PFS) or time to progression and overall survival (OS) (R = 0.84 and R = 0.83, respectively). **Nonetheless, significant differences in PFS only correlated with differences in OS in 3 out of 7 phase III studies identified. In these cases the hazard ratio for PFS was ≤0.6. Thus, the authors propose this threshold as a potential surrogate endpoint of OS in advanced HCC.**



Llovet *et al.*, 2019.
Progression-free survival: Endpoint for HCC trials?

HEPATOBILIARY ORGANOIDS

hiPSCs for generating organoids

Human induced pluripotent stem cell (hiPSC)-derived liver modelling systems have the potential to overcome the shortage of donors for clinical application and become a model for drug development. To date, few strategies have succeeded in generating a liver organoid with hepatobiliary structure from hiPSCs. **Here, Wu *et al.* reveal for the first time that functional hepatobiliary organoids have been generated from hiPSCs.** These findings are of major importance for future research on liver development and therapies in liver disease.

LIVER INJURY

Activated NOD1 attracts neutrophils in the liver, reactive cholangiocytes differentiate into proliferative hepatocytes

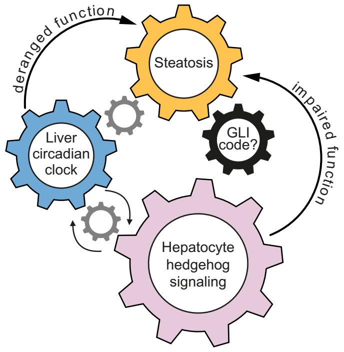
In liver transplantation, organ shortage leads to the use of marginal grafts that are more susceptible to ischemia-reperfusion (IR). Nucleotide-binding oligomerization domain 1 (NOD1) is a pattern-recognition receptor of the "noninflammasome" NLR (nucleotide-binding domain, leucine-rich repeat–containing) family. NOD1 protein is expressed in the cytosol to detect the presence of the bacterial pathogen-associated molecular pattern (PAMP), the peptidoglycan γ-D-glutamyl-mesodiaminopimelic acid. NOD1 can also be activated by mesolanthionine, another peptidoglycan-associated diamino-amino acid. PAMP detection by NOD1 is likely to result in a strong danger signal. NOD1 signalling is mediated by transcription factors, NF-κB and AP-1, resulting in the induction of a battery of chemokines, cytokines and defensins. NOD1 is an important modulator of neutrophil-induced liver injury, which occurs in IR. Lassailly *et al.* aimed to elucidate the role of NOD1 in liver IR, especially on the endothelium and hepatocyte. Leveraging the availability of *Nod1*-deficient mice, they show that **NOD1 regulates liver IR injury through induction of adhesion molecules and modulation of hepatocyte-neutrophil interactions.** Moreover, they show that nanoparticles loaded with a NOD1 antagonist reduce liver IR injury, suggesting new therapeutic approaches for preventing IR, especially in the context of liver transplantation.

Chronic liver diseases are characterised by expansion of the small immature cholangiocytes (*i.e.*, ductular reaction [DR]) which can differentiate into hepatocytes. Manco *et al.* investigated the kinetics of DR differentiation into hepatocytes as well as functional maturity, clonal expansion and resistance to stress of the newly formed hepatocytes in mice with long-term liver damage. **Here, they show that in chronic liver injury, DR-related cells differentiate into stress resistant-hepatocytes that repopulate the liver.**

NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Impaired cardiac function in NAFLD, role of hedgehog pathway in circadian rhythm

There is mounting evidence that patients with NAFLD have elevated cardiovascular risk, which negatively impacts long-term survival. However, the relationship between NAFLD and cardiac function is not well known. In this issue, Houghton *et al.* evaluated the relationship between liver fat and cardiac and autonomic function. Cardiovascular and autonomic function were assessed in individuals with NAFLD, steatosis and alcohol use (DAFLD) and in controls. **Both patients with NAFLD and DAFLD had impaired cardiac (*i.e.*, diastolic variability) and autonomic function** compared with controls. Importantly, the presence of hepatic steatosis and degree of liver fibrosis were associated with cardiac function. Interestingly, serum levels of inflammatory cytokines such as TNF-α were independently associated with autonomic function. This clinical study suggests that **both cardiac and autonomic impairment appear to**



Marbach-Breitrück *et al.*, 2019.
Role of Hedgehog pathway in circadian rhythm

be dependent on the level of liver fat and fibrosis staging, and to a lesser extent alcohol intake. Patients with NAFLD, especially those that also have excessive alcohol intake, should be monitored and advised to reduce cardiovascular risk.

Another interesting article in this issue uncovered novel mechanisms behind NAFLD. The mammalian circadian clock controls liver metabolism, while Hedgehog (Hh) signalling is known to regulate lipid metabolism. Marbach-Breitrück *et al.* investigated the crosstalk between hepatic Hh signalling and circadian rhythm. Hh signalling and the serum level of Indian Hh showed diurnal oscillations. **Depletion of the clock genes in hepatocytes and in mice resulted in abnormal expression of Hh genes and, conversely, disruption of Hh signalling resulted in altered expression of clock genes**. The clock/hedgehog module regulated rhythmicity of steatosis, in conditions of starvation or high-fat diet. This intriguing study shows an important role for Hh signalling in regulating hepatic lipid metabolism by modulating the circadian clock.

NOVEL GENETIC DIAGNOSIS

Role of whole-exome sequencing in the diagnosis of liver disease of unknown origin

The diagnosis of liver disease of unknown cause represents a clinical challenge. In this issue, Hakim *et al.* performed whole-exome sequencing (WES) in a series of 19 adults with unexplained liver disease despite comprehensive evaluation. **The authors identified 4 monogenic disorders in 5 unrelated adults**. The findings included deleterious heterozygous variant in *PPARG*, recessive mutations in *ABCB4*, a mitochondrial disorder due to a homozygous pathogenic variant in *NDUFB3* and a damaging heterozygous variant in *APOB*. In all these patients the findings guide some pathogenic-based therapy. This modern study strongly suggests that **genomic analysis may yield an actionable diagnosis in a substantial number of selected adult patients with chronic liver disease of unknown aetiology**. The use of WES represents a potential new diagnostic tool in clinical hepatology.

HEPATITIS B VIRUS (HBV) INFECTION

Augmenting HBV-specific CD4 T cells – it takes two to tango, finding those in need of treatment in sub-Saharan Africa, TDF resistance – a four-leaf clover

Resuscitation of exhausted or dysfunctional HBV-specific T cells by immune-checkpoint inhibition represents a logical approach in the attempt to accelerate immune-mediated elimination of chronic HBV infection. However, *in vitro* studies and early data from clinical trials did not show a clear-cut effect of programmed cell death (PD-1) immune-checkpoint inhibition on HBV-specific, T cell-mediated cytokine excretion and viral replication markers, suggesting that PD-1 blockade alone is probably insufficient to improve viral control. Jacobi *et al.* now tried to augment HBV-specific CD4 T cells from patients with HBeAg-negative chronic hepatitis B by targeting different immunological pathways. They showed that HBV-specific T cells strongly express PD-1, but also the co-stimulatory molecule OX40 (CD134), providing a strong rationale to study combined OX40 stimulation and PD-L1 blockade. **Functionally augmented HBV-specific CD4 T cells were observed only when combining OX40 stimulation and PD-L1 blockade**. These results may prove useful in designing novel immunotherapeutic interventions to contain or even cure chronic HBV infection.

In sub-Saharan Africa, 5–10% of the adult population is living with chronic hepatitis B. Finding those in need of treatment is of the utmost importance, but according to all current guidelines this necessitates a combined assessment of liver fibrosis stage, serum level of alanine aminotransferase (ALT), and HBV viral load – a diagnostic triumvirate that cannot be guaranteed in most low-income countries. In 2015, the World Health Organization (WHO) published guidelines for the prevention, care and treatment of chronic hepatitis B, with a special emphasis on resource-limited settings, including simplified criteria for treatment initiation based on clinical assessment, ALT-to-platelet ratio (APRI) and ALT, but not HBV viral load. As little is known about the accuracy and applicability of the WHO treatment criteria in sub-Saharan Africa, Desalegn *et al.* evaluated the concordance between the WHO 2015 and the EASL 2017 treatment eligibility criteria in one of the largest hepatitis B treatment programmes in Ethiopia. **The present study demonstrates that the WHO 2015 criteria for hepatitis B treatment indication failed to detect roughly half of the patients in need of treatment according to the EASL 2017 guidelines**. The authors conclude that the WHO guidelines are unsuitable in an African setting and require revisions that take local data from real-world hepatitis B cohorts in sub-Saharan Africa into account.

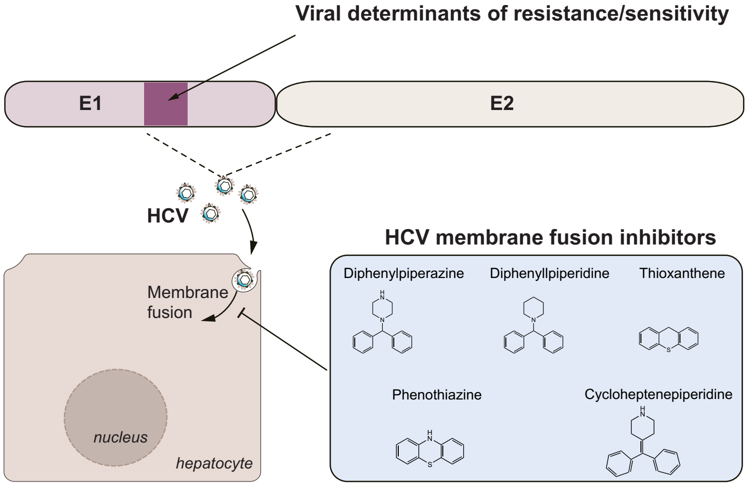
The nucleotide polymerase inhibitor tenofovir disoproxil fumarate (TDF) is highly effective in the treatment of chronic hepatitis B owing to its high-level genetic barrier against the development of resistance-associated variants. Indeed, strong evidence for a clinically relevant virologic failure caused by genotypic resistance to TDF is still missing. In this issue of the *Journal* Park *et al.* report on 2 patients with viral breakthrough during TDF treatment in whom in-depth genetic analyses of the HBV reverse transcriptase genes were performed and genetic variants further characterised by site-directed mutagenesis and drug susceptibility analyses. **The authors showed for the first time that to develop clinical resistance to TDF, the accumulation of at least 4 mutations are required,** and that this novel quadruple CYEI mutation (rtS106C, rtH126Y, rtD134E, and rtL269I) reduced tenofovir-susceptibility by more than 10-fold. After this first report of a potentially clinically relevant TDF-resistant variant, further studies are needed to evaluate the importance of these variants in larger patient cohorts with incomplete response to TDF, hereby also exploring how to treat them.

HEPATITIS C VIRUS (HCV) INFECTION

Trimming the peptides properly to clear acute HCV infection, unravelling mechanisms of HCV entry inhibition

Antigen presentation in the context of human leukocyte antigen (HLA) class 1 molecules depends on an optimised trimming of the peptides to an optimal length of usually 8 or 9 amino acids, which is mediated by the endoplasmic reticulum aminopeptidase 1 (ERAP1). Genetic variants of *ERAP1* have been linked to several HLA class I-associated autoinflammatory disorders. Longer fragments (10–12-mers) generated by discrete ERAP1 allotypes were associated with hypoactive forms, whereas shorter fragments (7–8-mers) were typically generated by hyperactive ones. The physiological relevance of these effects on the immune responses to exogenous antigens, like HCV infection, however, has not been addressed. In this study, Kemming *et al.* performed a comprehensive analysis of ERAP1 allotypes and virus-specific CD8$^+$ T cell responses in an HLA-B*27:05+ individual with acute hepatitis C who initially presented with favourable prerequisites for spontaneous viral clearance. Nevertheless, the patient progressed to chronic infection with low-level viremia. **The authors showed that 2 hypoactive allotypic ERAP1 variants modified the virus-specific CD8$^+$ T cell epitope repertoire *in vivo*, leading to altered immunodominance patterns**. This elegant study describes a new genetically determined immunologic mechanism that contributes to the failure of antiviral immunity after infection with HCV. This should also be explored in the context of other infections and their respective outcomes.

Targeting viral cell entry by specific entry inhibitors represents an attractive antiviral strategy with already proven efficacy in HIV-1 infection but which also gathered attention as a rescue approach for treating multidrug resistant HCV infection. Banda *et al.* investigated the mode of action of structurally related inhibitors of HCV entry, hereby showing that **molecules of the diphenylpiperazines, diphenylpiperidines, phenothiazines, thioxanthenes, and cycloheptenepiperidines chemotypes inhibit HCV infection via interference with membrane fusion**. The authors also defined viral determinants of resistance, mapped the viral target site of these molecules, and shed light on protein features of the envelop 1 protein that control pH dependent membrane fusion. These studies not only provide information on how to identify patients that may benefit from treatment with membrane fusion inhibitors but also set the stage for developing further improved inhibitors by unravelling the molecular mechanisms that control HCV membrane fusion.



Kemming *et al.*, 201
Unravelling mechanisms of HCV entry inhibitic

LIVER TRANSPLANTATION

Transplantation using HBV core antibody positive grafts

Mortality of patients on the waiting list for liver transplantation remains high and strategies to increase the donor pool are needed. At present, the safety of using hepatitis B anti-core antibody positive grafts is unknown. Wong *et al.* now describe the results of a retrospective study in a large number of patients in which they evaluated the outcomes of patients undergoing liver transplantation using organs from anti-HBcAb +ve and anti-HBcAb −ve donors. **Their data show that using anti-HBcAb +ve grafts was safe and the rate of development of *de novo* HBV infection was very low, especially if donors are receiving an entecavir based regimen.** These data are likely to result in a change in clinical practice and allow patients who are wait listed for liver transplantation access to more organs.

LIVER CANCER – CLINICAL

Washout for establishing tumour's nature in BCS, data integration including radiomic data to predict microvascular invasion

Distinguishing benign from malignant lesions can be challenging in patients with Budd-Chiari syndrome (BCS). To address this question, Van Wettere *et al.* evaluated the discriminating ability of washout in differentiating benign from malignant lesions in 49 patients with BCS. MRI images were reviewed by 2 radiologists blinded to the nature of the lesions. Patient and lesion characteristics were recorded, with a focus on washout on portal venous and/or delayed phases. **They now show that washout was observed in close to 1/3 of benign lesions leading to an unacceptably low specificity for the diagnosis of HCC. Moreover, they show that the non-invasive diagnostic criteria proposed by the AASLD/EASL for cirrhotic patients cannot be extrapolated to patients with BCS.**

Microvascular invasion (MVI) impairs surgical outcomes in patients with HCC. There is currently no single highly reliable factor to preoperatively predict MVI. Therefore, Xu *et al.* developed a computational approach integrating large-scale clinical and imaging modalities, in particular radiomic features from contrast-enhanced CT, to predict MVI and clinical outcomes in patients with HCC. **They reveal that a computational approach integrating large-scale clinical, radiologic and radiomic data was accurate in predicting MVI and disease clinical outcomes.** However, radiomics with current CT imaging analysis protocols do not show significant added value to radiographic scores.

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