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**FINAL**

**Richard Moreau*, Ramon Bataller, Thomas Berg, Sophie Lotersztajn, Jessica Zucman-Rossi, Rajiv Jalan**

**Richard Moreau*** at Centre de Recherche sur l'Inflammation (CRI), INSERM, Université Paris Diderot, Paris, France; DHU UNITY, Service d'Hépatologie, Hôpital Beaujon, Assistance Publique-Hôpitaux de Paris, Clichy, France; Laboratoire d'Excellence (Labex) Inflamex, COMUE Sorbonne Paris Cité, Paris, France; * Corresponding author *E-mail address*: richard.moreau@inserm.fr

**Ramon Bataller** at Division of Gastroenterology, Hepatology and Nutrition, University of Pittsburgh Medical Center, Pittsburgh, PA, USA.

**Thomas Berg** at Section Hepatology, Clinic for Gastroenterology and Rheumatology, University Hospital Leipzig, Leipzig, Germany.

**Sophie Lotersztajn** at Centre de Recherche sur l'Inflammation (CRI), INSERM, Université Paris Diderot, Paris, France

**Jessica Zucman-Rossi** at Inserm UMR-674; Génomique Fonctionnelle des Tumeurs Solides; IUH; Paris, France; Université Paris Descartes; Labex Immuno-oncology; Faculté de Médecine; Sorbonne Paris Cité; Paris, France.

**Rajiv Jalan** at Liver Failure Group, Institute for Liver and Digestive Health, University College London, Royal Free Hospital, UK

**SELECTION OF THE MONTH**

**Big Title: Progression free survival: End-point for HCC trials?**

**Small title: Transplantation using HBV core antibody positive grafts is safe**

**Augmenting HBV-specific CD4 T cells - it takes two to tango**

**TDF resistance - a four-leaved clover**

**Impaired cardiac function in NAFLD**

## HEPATOBILIARY ORGANOIDS

### hiPSCs for generating organoids

Human induced pluripotent stem cells (iPSC)-derived liver modeling 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 hepato-biliary structure from hiPSCs. **Here, Wu *et al.* reveal for the first time that functional hepato-biliary 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 attract neutrophils in the liver, reactive cholangiocytes differentiate into proliferative hepatocytes

In liver transplantation, organ shortage leads to use 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 signaling 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 such as in IR. **Lassailly *et al.*** aimed to elucidate the role of NOD1 in the liver IR, especially on the endothelium and hepatocyte. Leveraging the availability of *Nod1*-deficient mouse, they show that **NOD1 regulates liver IR injury through induction of adhesion molecules and modulation of hepatocytes-neutrophil interaction.** 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 characterized 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 NAFLD and DAFLD patients had impaired cardiac (i.e., diastolic variability) and autonomic function** when 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 impairments appear to be dependent on 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.

In another interesting article, in this issue, novel mechanisms of NAFLD have been uncovered. The mammalian circadian clock controls liver metabolism and the Hedgehog (Hh) signaling is known to regulate lipid metabolism. **Marbach-Breitrück *et al.*** investigated the crosstalk between hepatic Hh signaling and circadian rhythm. Hh signaling 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 signaling 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 signaling in regulating hepatic lipid metabolism homeostasis 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 etiology**. The use of whole-exome sequencing 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 for treatment - Lost in sub-Saharan Africa, TDF resistance - a four-leaved 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 also 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 HBeA-negative chronic hepatitis B by targeting different immunological pathways. They showed that HBV-specific T cells strongly express both, 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 with need of treatment is of upmost importance but necessitating - according to all current guidelines - 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 without HBV viral load. As little is known about the accuracy and applicability of the WHO treatment criteria in sub-Saharan Africa **Hanna *et al.*** evaluated the concordance between the WHO 2015 and the EASL 2017 treatment eligibility criteria in one of the largest hepatitis B treatment programs 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**. Authors conclude that the WHO guidelines might be unsuitable in an African setting, and need revisions which should 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 two 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 characterized 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 four mutations is 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 clinical relevant TDF-resistant variant, further studies are needed evaluating 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, unraveling mechanisms of HCV entry inhibition

Antigen presentation in the context of human leukocyte antigen (HLA) class 1 molecules depends on an optimized 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 and generating longer fragments (10–12mers) by discrete ERAP1 allotypes were associated with hypoactive forms whereas shorter fragments (7–8mers) 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 so far. 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 presenting initially with all favorable prerequisites to clear the virus spontaneously but nevertheless progressed to chronic infection with low-level viremia. **The authors showed that two 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 contributing to the failure of antiviral immunity after infection with HCV which should be also 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 unraveling the molecular mechanisms that control HCV membrane fusion.

## 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 is 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 graft was safe and the rate of development of *de novo* HBV infection was very low especially if they 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 tumor'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 of these patients. MR imaging (MRI) images were reviewed by two 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 hepatocellular carcinoma (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 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.