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FINAL

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NOTE: Unless specified, we used official gene names and symbols provided by HUGO Gene Nomenclature Committee (www.genenames.org) and protein names and symbols provided by Uniprot (www.uniprot.org).

SELECTION OF THE MONTH

Big Title: Tea and coffee are good for the liver

Small titles: Novel PET tracer to visualize biliary tree

A potential high-risk mutation for HBV-infected individuals

Global epidemiology of HCV subtypes and resistance-associated substitutions

Trends in HCV incidence among HIV-positive MSM

METABOLIC LIVER DISEASES

Cyclic AMP-dependent activating transcription factor ATF-3, beta-catenin and CD44: new molecular drivers of NASH and iron-overload induced liver injury

Cyclic AMP-dependent activating transcription factor 3 ATF-3 (encoded by *ATF3* and hereafter called ATF-3) plays a role in type 2 diabetes and insulin resistance. In this issue of the Journal, Kim *et al.* studied its role in NAFLD. ATF-3 was highly expressed in the liver from patients with NAFLD and in Zucker diabetic fatty (ZDF) rats. Insulin resistance and hepatic steatosis were associated with increased ATF-3 expression and decreased fatty acid oxidation. Importantly, ***Atf3* suppression ameliorated glucose intolerance and inflammatory responses in ZDF rats**. In patients with NAFLD, ATF-3 expression correlated with surrogate markers of type 2 diabetes and hepatic inflammation. Collectively, the results of this translational study suggest a role for ATF-3 in insulin resistance and hepatic inflammation in patients with NAFLD and represents a novel target for therapy. In another interesting study, Preziosi *et al.* studied the link between beta-catenin (encoded by *Ctnnb1* in mice), iron overload and liver injury. *Ctnnb1*-knockout mice were exposed to an iron-overload diet in the presence or absence of NAC, an antioxidant agent. ***Ctnnb1*-deficient mice expose to iron exhibited remarkable inflammation, fibrosis and occurrence of occasional HCC**. Interestingly, antioxidant therapy ameliorated this changes, Addition of **NAC to drinking water protected mice from liver injury** and prevented the activation of pro-inflammatory signaling pathways. This study reveals a new role for beta-catenin and suggests that antioxidants may have a protective role against iron-induced liver injury. Finally, another study from this issue analyzes the role of CD44, a receptor that regulates adipose tissue inflammation in obesity. Patouraux *et al.* provide elegant evidence that mice deficient in *CD44 antigen* (*Cd44*) are resistant to develop liver inflammation and fibrosis after a fibrogenic insult. Interestingly, *Cd44* deficiency enhanced the M2 polarization and strongly decreased the activation of macrophages by lipopolysaccharide, hepatocyte damage-associated molecular patterns, and saturated fatty-acids. Neutralization of CD44 antigen protein decreased macrophage infiltration. Importantly, in NASH patients hepatic CD44 and soluble CD44 antigen in serum was increased and correlated with the severity of liver injury. **This study suggests that CD44 is a biomarker and a molecular player in NASH by regulating macrophage infiltration and polarization**.

LIVER FIBROSIS

Coffee and herbal tea consumption is protective of liver stiffness in the general population

Coffee and tea have been proposed to have beneficial effects on liver diseases including HCC development. Alferink *et al.* analyzed the participants who underwent transient elastography, ultrasound and completed a food frequency questionnaire from the Rotterdam study, a large prospective population-based cohort. The authors included 2,424 participants of whom 5 % had LSM ≥ 8.0kPa and 34% steatosis. **Proportion of LSM ≥ 8.0kPa decreased with higher coffee consumption.** Amongst tea consumers, **only herbal tea consumers (36%) had lower log-transformed transient elastography levels**. Subtypes of tea were associated with steatosis in univariate but not multivariable analysis. This important epidemiological study suggests that in the general population, frequent coffee and herbal tea consumption are inversely related with liver stiffness but not steatosis. This results should be confirmed in prospective longitudinal studies.

HEPATITIS C VIRUS (HCV) INFECTION

Global epidemiology of HCV subtypes and resistance-associated substitutions, next generation pangenotypic DAA combination, trends in HCV incidence among HIV-positive MSM, scavenger receptor class B member 1 genetic variants modulate HCV infection

HCV genotypes and subtypes as well as the presence of baseline resistance-associated substitutions (RASs) represent key viral determinants for the selection of direct-acting antiviral (DAA) treatment regimens. The study by Welzel *et al.* is the first comprehensive global molecular epidemiology analysis of HCV subtypes and subtype-specific RASs. The analyses were performed by both, INNO-LiPA and sequencing on 12,615 patient samples from 28 different countries across five geographic regions. Whereas genotyping concordance was high between both methods, the INNO-LiPA had significant limitations in subtyping especially of the types 2, 3, 4 and 6. **The observed variations in regional subtype epidemiology but also subtype-specific RAS prevalence may have crucial implications when designing future global HCV treatment strategies**.

Aiming for antiviral regimens being highly effective irrespective of the HCV type/subtype and presence of baseline resistance-associated variants was the rationale behind second generation DAA drug development. The NS3/4A protease

inhibitor glecaprevir and NS5A inhibitors pibrentasvir (G/P) have potent antiviral activity *in vitro* against all six major HCV genotypes with a high barrier to the selection of common viral variants with resistance-associated substitutions. In this issue of the Journal, Kwo *et al.* present results from two phase 2 studies (SURVEYOR-I and SURVEYOR-II, parts 1 and 2) designed to evaluate the efficacy and safety of various doses of G/P with or without ribavirin for the treatment of non-cirrhotic patients with HCV genotype 1, 2, 3, 4, 5, or 6 infection. Eight-week treatment with the dosage-optimized regimen yielded SVR rates of 97-98% in HCV type 1-3 infection (with no virologic relapses), and 100% of type 4-6 infected patients achieved SVR after a 12 week course. **These studies demonstrate the ability of the G/P combination to treat all six major HCV genotypes with a single ribavirin-free regimen regardless of baseline resistance-associated polymorphisms, and with treatment durations as short as eight weeks in populations without cirrhosis**.

HCV infection continues to spread among HIV-positive men who have sex with men (MSM), especially among younger individuals. This cohort study from Europe, Australia and Canada aims at estimating trends in HCV incidence among HIV-positive MSM with well-estimated dates of HIV seroconversion from the CASCADE Collaboration, but also assessed the association between HCV incidence and geographical region, age, HIV RNA and CD4 count. Van Santen et al. showed that **HCV incidence significantly increased after 1990 and there was no evidence of a decline in recent years. However, trends seem to differ by geographical region.** While HCV incidence appears to have stabilized in Western Europe, and remained stable in Southern Europe, an increase in HCV incidence was observed in Northern Europe. Interestingly, a higher HIV RNA and younger age were associated with a higher HCV incidence. This large-scale study adds important data to the current body of evidence regarding HCV trends among HIV-positive MSM and on the association of HIV-related factors with HCV infection.

Scavenger receptor class B member 1 (SRB1) is one of four hepatocyte surface expressed molecules regarded as essential for HCV host cell entry. Physiologically, SRB1 functions as a multiligand receptor that binds lipoproteins. Numerous studies indicate that genetic variations in the *SCARB1* gene that encodes SRB1 are associated with clinical phenotypes, yet their impact on HCV infection is incompletely understood. Westhaus *et al.* performed the most comprehensive study to date evaluating the impact *SCARB1* genetic variants on the molecular biology of HCV and

the clinical course of hepatitis C. **They found evidence that both coding and non-coding genetic variants affect the HCV replication cycle as well as clinically relevant HCV-related parameters in HCV infected individuals.** Moreover, genetic deletion of *SCARB1* from an HCV permissive cell line results in a markedly reduced susceptibility to HCV infection. Overall, this important study add to our understanding on the role of genetic host factors in modulating the disease characteristics herby explaining - at least in part - the remarkable high degree of inter-individual variations seen in the course of HCV infection.

HEPATITIS B VIRUS (HBV) INFECTION

A potential high-risk mutation for HBV-infected individuals selected during TDF and ETV combination therapy

Long-term treatment with antiviral drugs carries the risk of selecting mutations in the HBV polymerase. Tacke *et al.* report two cases of patients with insufficient response to dual tenofovir (TDF) and entecavir (ETV) therapy in whom ultra-deep pyrosequencing analyses revealed the selection of the rtS78T polymerase mutation. **Interestingly, the rtS78T polymerase mutation causes enhanced viral replication, and reduced susceptibility to ETV and TDF *in vitro*, and creates a premature stop codon at sC69 which causes truncation of the hepatitis B surface protein (HBsAg), thereby deleting almost the entire small HBsAg**. These interesting findings will stimulate further research in order to define the role of this pontential high-risk mutation with respect to nucleos(t)ide-analog treatment failure and HBV-related carcinogenesis.

BILIARY IMAGING

Development of a novel PET tracer to visualize the biliary tree

Bile acid flow and signaling is fast becoming important target for new drug development. Imaging techniques aimed at evaluating bile flow are limited. The study by Ørntoft *et al.* aimed to use a radiolabelled conjugated bile acid tracer [N- methyl-^{11}C] cholylsarcosine (^{11}C-CSar), that they developed for quantification of hepatobiliary secretion in healthy volunteers and patients with cholestatic liver diseases using PET scanning. **The authors provide the exciting novel method and data that allows accurate quantification of bile acid kinetics that will be revolutionary in providing better understanding of the pathophysiology of**

cholestatic diseases.

BILIRUBIN HEPATOXICITY

Bilirubin breakdown products, Z-BOX A and Z-BOX B are hepatotoxic

It is well known that in conditions of severe cholestasis, bilirubin can have cytotoxic effects but the mechanism is unclear. Under conditions of oxidative stress, unconjugated bilirubin is oxidized to higher-order degradation products such as the major bilirubin oxidation end products (BOXes) but its role in mediating further liver injury is unknown. **The paper from Seidel *et al.* provides measurements of these metabolites in health and in patients with liver failure. They show for the first time that there are detectable levels of these Z-BOXes in healthy individuals and markedly increased in patients with liver failure. They also show that these substances can induce dose dependent cytotoxicity and glutathione depletion.** These novel data provide exciting new insight into the pathogenesis of liver injury and potential therapeutic target.

HEPATOCELLULAR CARCINOMA (HCC)

OGT uses palmitate and ER stress to promote HCC in NAFLD, targeted deep sequencing for circumventing tumor heterogeneity, tumor-associated microparticles as biomarkers for cancer, global HCC epidemiology

O-linked N-acetylglucosamine (GlcNAc) transferase (symbol *OGT*) encodes a glycosyl transferase involved in metabolism reprogramming. *OGT*, has been shown to be upregulated in tissues non-alcoholic fatty liver disease (NAFLD)-associated-HCC (NAFLD-HCC). Xu *et al.* here address the oncogenic role of *OGT* in NAFLD-HCC by investigating the effects of *Ogt* gain- or loss- of function in vitro and in nude mice. **They reveal that *OGT* plays an oncogenic role in NAFLD-HCC through regulating palmitic acid and inducing ER stress, consequently activating oncogenic JNK/c-jun/AP-1 and NF-κB cascades.**

In HCC, intratumoral heterogeneity challenges the identification of mutations which may be targets for therapies. Using whole-exome sequencing and targeted-deep sequencing (TDS), Huang *et al.* now show that **TDS of single tumor specimen could largely circumvent intratumoral heterogeneity and uncover actionable mutations indicative of target therapy in HCC.**

It has been shown that tumor-associated microparticles (taMPs) bearing annexin V, Ep-CAM (for epithelial cell adhesion molecule), and CD147 (recommended name, basigin) can be used as biomarkers for cancer. Julich-Haertel *et al.* investigated taMPs in the context of HCC and cholangiocarcinoma (CCA). They enrolled a large series of patients with liver cancer (HCC or CCA), cirrhosis (without cancer), and control subjects, and performed fluorescence-activated cell scanning to detect taMPs in the serum. They show that **taMPs bearing Annexin V, EpCAM, and ASGPR 1 can be considered as a novel biomarker of HCC and CCA liquid biopsy that permit a non-invasive assessment of the presence and possibly the extent of these cancers in patients with advanced liver diseases.** Of note, ASGPR 1 is the short name for asialoglycoprotein receptor 1 (encoded by *ASGR1,* belonging to the C-type lectin domain containing [CLEC] gene family).

In this issue of the Journal, Bertuccio *et al.* report the results of their updating of global trends in HCC mortality to 2014, and predict trends in rates in the European Union, Unites-States of American, and Japan to 2020, using data from the World Health Organization database. **They show that HCC mortality falls in Eastern Asia and Southern Europe mainly because of the control of HBV and HCV virus infections. There is an unfavorable trends in other areas of the world due to HCV (and HBV) epidemics between the 60s and 80s, changes in alcohol consumption, and increased overweight/obesity, and consequently diabetes.** Improvements in the management of cirrhosis and in HCC diagnosis and treatment account for part of the mortality trends worldwide.