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From the Editor's Desk...

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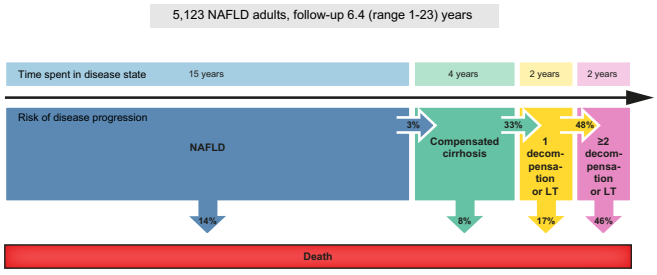
SELECTION OF THE MONTH

Clinical course of NAFLD and the implications for clinical trial design

In non-alcoholic fatty liver disease (NAFLD), the risk of disease progression and the incidence of hard clinical outcomes are not well established at the population level. **Allen *et al.*** performed a study including 5,123 adults diagnosed with NAFLD between 1996 and 2016 in Olmsted county, Minnesota, who were followed for a median of 6.4 years for liver-related outcomes and death. The study found that **the risk of progression from NAFLD to cirrhosis was 3% in 15 years**, **from compensated cirrhosis to first decompensation was 33% in 4 years (8%/year), and from first decompensation to subsequent decompensation was 47% in 2 years.** Only 6% of the 575 deaths were liver-related but the cohort included all-comers with NAFLD and not only patients with steatohepatitis. Albumin, bilirubin, non-bleeding oesophageal varices and diabetes were independent predictors of decompensation. Finally, based on these observed rates of disease progression, the authors calculated that therapeutic trials in compensated cirrhosis would require minimum enrolment of 2,886 participants followed over 2 years to detect at least a 15% relative decrease in liver endpoints, which suggests that current trials are severely underpowered.



Estimated sample sizes for clinical trials in NASH cirrhosis

Disease state		Compensated cirrhosis	Decompensated cirrhosis
Outcomes (any of)		Hepatic decompensation, HCC, transplantation, death	Liver transplantation, HCC, death
Probability of events		42% in 4 years	65% in 2 years
Sample size based on anticipated	15%	2,886	1,356
decrease in incidence in the	25%	882	420
intervention group versus placebo	35%	408	198

Allen *et al.*, 2022.
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EXPERIMENTAL AND TRANSLATIONAL HEPATOLOGY

Farnesoid X receptor differentiates hybrid intestinal-liver stem cells towards functional hepatocytes

Induced pluripotent stem cells (iPSCs) have emerged as a cellular source of hepatocyte-like cells for regenerative medicine or drug testing, but some differences between iPSC-derived and mature hepatocytes remain. Using sophisticated differentiation protocols and innovative, single-cell-based technologies, **Nell, Kattler, Feuerborn *et al.*** compared mature, foetal and iPSC-derived human hepatocytes and found that iPSC-derived liver cells show hepatic and intestinal features. Stimulating the farnesoid X receptor (FXR) via pharmacological FXR agonists or lentiviral expression **favoured the hepatocyte-like maturation of iPSC-derived cells and suppressed intestinal traits.** These findings are expected to advance the iPSC-derived generation of hepatocytes for cellular models and regenerative medicine.

Scar-associated infiltrating TREM2+ macrophages limit steatohepatitis and liver fibrosis

Recent single-cell RNA sequencing studies have identified that a specific population of TREM2+ macrophages are localised around fibrotic scars in cirrhosis. **Hendrikx, Porsch *et al.*** have employed mouse models of steatohepatitis and fibrosis to understand the origin and function of these TREM2+ macrophages. These cells are bone-marrow derived and infiltrate the liver, while TREM2 is functionally involved in lipid handling and the antifibrotic actions of macrophages. Intriguingly, **soluble TREM2 levels in the blood seem to mirror the dynamics of liver-infiltrating TREM2+ macrophages**, indicating the potential value of this measure as a biomarker in patients with progressive NAFLD.

New insights into the immune pathogenesis of biliary atresia: Targeting CD177+ neutrophils

Biliary atresia is a neonatal disease associated with poor prognosis, if not treated with Kasai portoenterostomy or liver transplantation. **Zhang, Su, Fu *et al.*** investigated the contribution of immune cells to the pathogenesis of biliary atresia, using patient samples and a mouse model of the disease. They report **increased hepatic and circulating populations of CD177-expressing neutrophils, which aggravate biliary atresia in mice** by releasing neutrophil extracellular traps (NETs) causing apoptosis of biliary epithelial cells. In a pilot study, 6 patients with biliary atresia received N-acetylcysteine infusions before surgery, which inhibits NET formation and reduced the CD177+ cell numbers. These data indicate that immune pathology contributes to biliary atresia and may be therapeutically targeted.

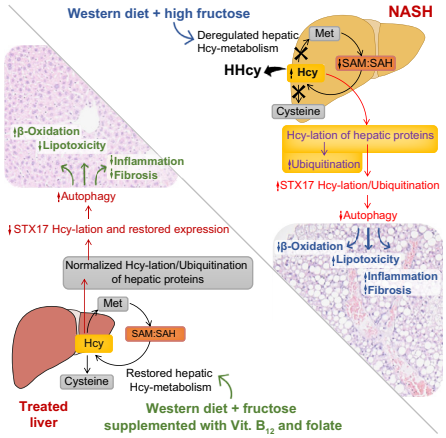
Intrahepatic tissue-resident memory T cells represent a potential therapeutic target in PBC

The E2 component of mitochondrial pyruvate dehydrogenase complex (PDC-E2) is a common autoantigen in primary biliary cholangitis (PBC). **Huang, Lyu,**

Qian *et al.* identify CD69+CD103+CD8+ tissue-resident memory T (T_{RM}) cells as the dominant intrahepatic population of PDC-E2-specific CD8 T lymphocytes in PBC that have cytotoxic effects on cholangiocytes in a 3D organoid co-culture system. Pharmacological blockade or genetic deletion of NUDT1, an oxidised purine nucleoside triphosphatase, eliminated CD103+ T_{RM} cells and alleviated cholangitis in a PBC mouse model, indicating that these **liver-infiltrating pathogenic CD103+ T_{RM} cells could represent a novel therapeutic target in PBC.**

Hyperhomocysteinemia is a driver and therapeutic target in NASH

Hyperhomocysteinemia (high serum homocysteine levels) is considered a metabolic disorder related to genetic risk (*e.g. MTHFR* mutations) or low dietary intake of folate or vitamin B12. **Tripathi, Singh *et al.*** investigated the link between hyperhomocysteinemia and non-alcoholic steatohepatitis (NASH). In patients, hyperhomocysteinemia correlates with NASH progression. In experimental models, **high homocysteine levels exacerbate NASH via homocysteinylation of the key autophagy protein syntaxin 17 in the liver**. Vitamin B12 and folate supplementation restored autophagy and reduced NASH progression in mice, indicating that this simple nutritional intervention could be therapeutically relevant for patients with NASH.



Tripathi, Singh *et al.*, 2022. Hyperhomocysteinemia is a driver and therapeutic target in NASH.

NAFLD/NASH

Digital pathology with artificial intelligence analyses provides greater insights into treatment-induced fibrosis regression in NASH

The landmark paper by **Naoumov *et al.*** is an example of advances that digital pathology may bring over conventional pathology for the interpretation of histological changes induced by therapy. The authors studied unstained sections from 198 liver biopsies from a subgroup of 99 patients (paired: baseline and end-of-treatment) from a phase IIb trial for NASH who received placebo or 2 doses of tropifexor for 48 weeks. When assessed by conventional pathology, the trial did not show significant differences between tropifexor and placebo. Fibrosis, steatosis and hepatocyte ballooning were quantified using second harmonic generation/two-photon excitation fluorescence. Unlike conventional microscopy, digital analyses revealed a treatment-associated reduction of overall liver fibrosis. It also had the ability to show regressive changes in septa morphology and reduction in septa parameters particularly in F3 patients, who were judged as 'unchanged' with conventional pathological scoring. Patients with greater steatosis reduction also had the greatest reduction in perisinusoidal fibrosis. This study is important because **it reveals anti-fibrotic effects which were not captured by the NASH CRN scoring system and conventional microscopy**. As such, it may change the paradigm of how we assess histological changes, particularly in shorter term clinical trials when conventional pathology may not be sensitive enough to detect histological changes, particularly for liver fibrosis.

VIRAL HEPATITIS

HBV-specific CD4 T-cell responses differentiate functional cure from chronic surface antigen$^+$ infection

Sustained functional cure of chronic HBV occurs in few individuals with or without antiviral treatment and a better definition of what mediates functional cure is essential to improve immunotherapeutic strategies. **Ruben Hoogeveen *et al.*** aimed to compare HBV-specific T-cell responses in patients with different degrees of viral control. They obtained blood from 124 HBV-infected individuals, including acute self-limiting HBV infection, chronic infection, and chronic infection with functional cure. **ELISpot screening readily identified HBV-specific CD4 and CD8 T-cell responses in acute resolving infection compared with more limited reactivity in chronic infection.** Applying more sensitive assays revealed higher frequencies of functional HBV-specific CD4 T cells, but not CD8 T cells, in functional cure compared to chronic infection. The authors conclude that immunotherapeutic approaches to induce HBV functional cure should aim to improve CD4 T-cell responses.

Combination treatments including the small-interfering RNA JNJ-3989 induce rapid and sometimes prolonged viral responses in patients with CHB

RNA interference therapy has been shown to reduce HBsAg levels in preclinical models, which could confer functional cure in patients with chronic hepatitis B (CHB). In a phase II trial led by **M.-F. Yuen *et al.*** treatment-naïve and nucleos(t)ide analogue-suppressed patients received 3 subcutaneous doses of the small-interfering RNA JNJ-3989 every week (100, 200 or 300 mg), 2 weeks (100 mg) or 4 weeks (Q4W; 25, 50, 100, 200, 300 or 400 mg), or JNJ-3989 Q4W (200 mg) plus the capsid assembly modulator JNJ-6379 250 mg daily for 12 weeks. All treatments were well tolerated. HBsAg reductions were similar for HBeAg-positive and -negative patients. HBsAg reductions were lower with the 25 mg and 50 mg doses. **HBsAg reductions ≥1 $\log_{10}$ IU/ml from baseline persisted in 38% of patients 336 days after the last JNJ-3989 dose**.

Safety and efficacy of vebicorvir administered with entecavir in treatment-naïve patients with chronic HBV infection

Vebicorvir (VBR) is an investigational core inhibitor which interferes with multiple aspects of HBV replication. In a phase II trial, **Mark Sulkowski *et al.*** evaluated the efficacy and safety of 300 mg VBR (or

placebo) once daily in combination with entecavir (ETV) in treatment-naïve, HBeAg-positive, non-cirrhotic patients with chronic hepatitis B. VBR was well tolerated. At week 24, VBR+ETV led to a greater reduction from Baseline in log_{10} IU/ml HBV DNA (−5.33) *vs.* placebo+ETV (−4.20). **Greater mean reductions in pregenomic RNA were observed at week 12 and 24 in patients receiving VBR+ETV *vs.* placebo+ETV, while changes in viral antigens were similar in both groups**.

Influence of hepatitis C viral parameters on pregnancy complications and risk of mother-to-child transmission

Data on HCV in pregnancy are needed to determine the association of HCV viremia with adverse pregnancy outcomes and mother-to-child transmission. In a retrospective cohort study, **Tatyana Kushner *et al.*** identified 2,170 pregnancies in 1,636 women who were HCV RNA positive prior to pregnancy, 1,780 pregnancies were HCV RNA positive during pregnancy. **Pregnancies with positive HCV RNA were more likely to have preterm delivery (18% *vs.* 12%), intrahepatic cholestasis of pregnancy (4% *vs.* <2%), and postpartum haemorrhage (9% *vs.* 5%), and less likely to have gestational diabetes (6% *vs.* 10%) than those with resolved infection.** Only 511 (29%) infants had screening consistent with guidelines after birth. There was an estimated 3.5% risk of mother-to-child transmission, a significant risk factor was an HCV RNA level above 6 log10 IU/ml.

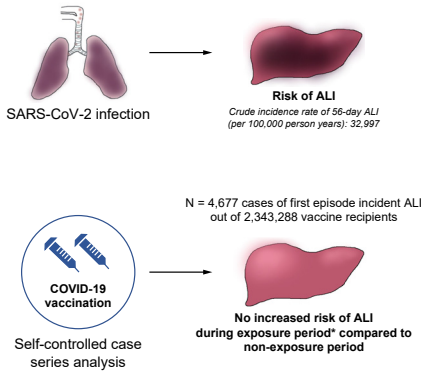
CIRRHOSIS AND LIVER FAILURE

Combination of G-CSF and a TLR4 inhibitor reduce inflammation and promote regeneration in a mouse model of ACLF

Acute-on-chronic liver failure (ACLF) is characterised by systemic and hepatic inflammation, non-apoptotic cell death and defective hepatic regeneration and carries a very high 90-day mortality rate. In an effort to explore new therapeutic modalities, **Engelmann *et al.*** tested the combination of granulocyte-colony stimulating factor (G-CSF) and TAK-242, a toll-like receptor-4 (TLR4) antagonist in 2 different murine models of ACLF (carbon tetrachloride with lipopolysaccharide [LPS model] or galactosamine [GalN model]). In the LPS model, GCSF alone increased mortality but in combination with TAK-242 it abrogated mortality and significantly reduced liver cell death, macrophage infiltration and inflammation. In the GalN model, **the combination was significantly more effective than each agent used alone.** G-CSF treatment resulted in the activation of the pro-regenerative and anti-apoptotic STAT3 pathway while TAK-242 reduced p21 over-expression suggesting reversal of hepatic senescence. The coadministration resulted in a significant increase of hepatocyte regeneration. This study provides the biological rationale for this particular combination therapy for the treatment of ACLF.

Risk of acute liver injury following the mRNA (BNT162b2) and inactivated (CoronaVac) COVID-19 vaccines

In this study, **Wong, Mak and co-workers** aimed to evaluate the risk of acute liver injury (ALI) following COVID-19 vaccines (BNT162b2 or CoronaVac). A modified self-controlled case series analysis was performed using the vaccination records in Hong Kong. Incidence rate ratios for ALI in the 56-day period following first and second doses of COVID-19 vaccines in comparison to the non-exposure period were estimated and compared to the ALI risk in patients with SARS-CoV-2 infection. Among the 2,343,288 COVID-19 vaccine recipients who were at risk, 4,677 patients had the first incident ALI from 23rd February 2021 to 30th September 2021. The number of ALI within 56 days after the first and second dose of vaccination were 307 and 521 for BNT162b2, and 304 and 474 for CoronaVac, respectively, compared to 32,997 ALI cases among patients within 56 days of SARS-CoV-2 infection. Compared to the non-exposure period, no increased risk was observed in the 56-day risk period for first and second dose of BNT162b2. Of all ALI cases following COVID-19 vaccination, no severe or fatal cases were detected. Thus, **there was no evidence of an increased risk of ALI associated with BNT162b2 or CoronaVac vaccination** and the benefit of mass vaccination seems to far outweigh the ALI risk from vaccination.



Wong, Mak, *et al.*, 2022.
Risk of ALI following COVID-19 vaccination

Third dose of COVID-19 mRNA vaccine appears to overcome vaccine hyporesponsiveness in patients with cirrhosis

In this retrospective study, **John and co-workers** compared outcomes in patients with cirrhosis who received 3 doses of either Pfizer BNT162b2 mRNA or Moderna mRNA-1273 vaccines to a propensity-matched control group of patients at similar risk of infection, who received 2 doses. Participants who received 3 doses of the vaccine were matched with 13,041 controls who received 2 doses. Receipt of the third dose of a COVID-19 mRNA vaccine was associated with an 80.7% reduction in COVID-19, an 80.4% reduction in symptomatic COVID-19, an 80% reduction in moderate/severe or critical COVID-19, a 100% reduction in severe or critical COVID-19, and a 100% reduction in COVID-19-related death. **The magnitude of reduction in COVID-19 was greater with the third dose of BNT 162b2 than mRNA-1273 and among participants with compensated rather than decompensated cirrhosis.** Thus, the administration of a third dose of a COVID-19 mRNA vaccine was associated with a more significant reduction in COVID-19 in patients with cirrhosis than reported in the general population,

suggesting that the third dose can overcome vaccine hyporesponsiveness in this population.

BILIARY CANCER

Targeting hyperactivated Tregs to improve antitumour immunity in cholangiocarcinoma

Intrahepatic cholangiocarcinoma (iCCA) was considered resistant to immunotherapies until recently, when the addition of an immune checkpoint inhibitor to chemotherapy resulted in improved survival. Using single-cell technologies, **Alvisi, Termanini *et al.*** studied the characteristics of leukocytes isolated from the tumour, the adjacent tumour-free tissue and peripheral blood mononuclear cells from surgically resected patients. Hyperactivated CD4+ regulatory T cells (Tregs) were abundant in the tumour infiltrate. Gene expression profile analysis showed that **T cells and natural killer cells undergo specific transcriptional changes inside the tumour which lead to enhanced immunosuppression by CD4+ Tregs**. These cells strongly expressed mesenchyme homeobox 1 or MEOX1, a transcription factor with poorly known function, and such overexpression enabled the reprogramming of circulating Tregs, which acquired the transcriptional and epigenetic landscape of tumour-infiltrating Tregs. The abundance of the MEOX1-dependent gene program in Tregs was strongly associated with poor prognosis in a large cohort of patients with iCCA. Ultimately, **the study opens the possibility of using these findings to improve antitumour immunity in iCCA**.

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