'Targeting the gut-liver axis in liver disease'

Clinical Trial Watch

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Key words: liver-gut-axis, bacterial translocation, liver injury, fibrosis, cirrhosis, bile acids, microbiome, incretines, pre-, probiotics, faecal microbial transplantation

“Always trust your gut – it knows what your head and liver has not yet figured out”

Summary

See suggestion Duncan

Key points:

-The gut-liver-axis has matured from a pathophysiological concept with experimental data on mechanisms and intrahepatic effects to clinical trials on therapeutic and preventive measures aiming to improve prognosis of multiple chronic liver diseases.

- Absorbable antibiotics should not be used to target the microbiome whereas non-absorbable antibiotics, e.g. rifaximin are well suited to do so but most likely exert many effects independent from their bactericidal action.

- Modulation of the microbiome by pre-/pro-and synbiotics can deliver significant positive hepatic effects without much concern on major side-effects. In contrast, safety and efficacy of fecal microbial transplantation or adsorbents are so far less clear.

- Bile acids (BA) and associated signaling, mainly via the nuclear farnesoid X receptor (FXR) are key players in the gut-liver-axis affecting intestinal barrier function as well as lipid and glucose metabolism. Hence, multiple promising different pharmacological FXR-modulators are currently tested in various liver diseases.

- Incretins and recombinant enterokines potently modulate food intake, nutrient absorption and metabolism by which first promising therapeutic results in metabolic liver disease are observed.

Introduction [1]

Open to the outer environment, the gut harbours a microbiome containing several-fold more genetic material than the human genome. It produces a myriad of metabolites, as well as hormones and peptides. The liver is at the nexus between this vast source of nutrients, toxins and hormones, and the rest of the body. Unsurprisingly, in experimental models and *in vitro* systems, the liver-gut-axis has been demonstrated to contribute to the pathogenesis of most liver diseases, such as alcoholic and non-alcoholic fatty liver disease (NAFLD), steatohepatitis

(NASH), cholestatic liver diseases, hepatocellular carcinoma (HCC), acute-on-chronic liver failure, progression to fibrosis/cirrhosis and complications of cirrhosis. Therapeutic approaches can be grouped into modulation of the microbiota, the bile acid (BA) pool and/or its signalling, gut lumen adsorptive strategies, bariatric procedures, incretins and miscellaneous (*e.g*. prokinetics). However, investigations in humans are key. Thus, this article will highlight the most recent human studies and clinical trials targeting the liver-gut-axis. A list of ongoing (not yet published) trials is presented in Table 1. Moreover, we take the liberty of encouraging clinical trials on unestablished concepts

Background: Pathophysiology [1]

"*Whatever comes from the gut enters the liver; the portal circulation is the afferent and the biliary tree is the efferent of the gut-liver-axis*" (Fig.1): The liver is the recipient and filter of nutrients, bacterial products/toxins and metabolites from the intestine. We are becoming increasingly aware of interactions between the gut, liver, immune system and metabolism. For instance, the term "metabolic endotoxemia" has been coined since Cani *et al.* discovered that the microbiome is involved in the onset of insulin resistance, low-grade inflammation and diabetes[1]. This stems from the observation that constituents of gram-negative bacteria, which are present in the blood stream at very low levels because of translocation from the gut, could trigger inflammation and alter glucose metabolism[1]. A complete list and overview of all the different components or metabolic products of gut bacteria, products, intestinal hormones, peptides and gut-derived neurotransmitters are beyond the scope of this article. Therefore, this article focusses on pathogen/microbe-associated molecular patterns (P/MAMPs), of which bacterial lipopolysaccharides (LPS), peptidoglycans, flagellin and bacterial DNA are prototypical.

The immune system recognises P/MAMPs via pattern recognition receptors, such as toll-like-receptors and nucleotide-binding oligomerisation domain like receptors (NLR). To oversimplify, an increased inflow and/or susceptibility to P/MAMPs via pathological bacterial translocation induces a pro-inflammatory intrahepatic milieu driven by mononuclear cell-released cytokines such as tumour necrosis factor (TNF), interleukin (IL)-1, and IL-6. In fact, lack of tolerance to LPS in human liver slices from cirrhotic livers, compared to healthy controls, creates a comparable inflammatory response (IL-1b, IL-6 and -8), induced by LPS

challenge[2]. This intrahepatic P/MAMP-aemia and pro-inflammatory milieu induces the production of reactive oxygen species by parenchymal and non-parenchymal liver cells, promoting liver injury and fibrosis. However, the liver is not only exposed to gut-derived P/MAMPs, but also absorbed products derived from bacterial metabolism and/or the interaction of the microbiota with the diet. Such products include ethanol, acetaldehyde, trimethylamine and short chain and free fatty acids, which can aggravate and perpetuate liver damage and fibrogenesis and contribute to steatosis/hepatitis. For instance, upregulated palmitic acid absorption with altered intestinal transporters has recently been reported in early NASH[3]. It has also been shown to associate with clinicopathological features[3]. Finally, adipokines and cytokines derived from visceral fat can contribute to intrahepatic inflammation[4] and have even been proposed to determine HCC risk[5]. In fact, at AASLD 2016 central fat (estimated as waist circumference and/or –hip ratio) was proposed to predict the presence of NASH more accurately than BM[I]{Balakrishnan, 2017 #839}, being an even stronger risk factor in lean patients than in the obese[{Fracanzani, 2017 #840},8]. Disruptions of the intestinal barrier, leading to increased permeability, qualitatively and quantitatively influence the P/MAMPs and bacterial metabolites to which the liver is exposed. Consequently, access to the portal-venous circulation is determined by the gut-vascular barrier, which has recently been characterised[9]. Indeed, the opening of the gut-vascular barrier is likely to be as fundamental to gut-liver-axis pathophysiology as the translocation process at the epithelial layer, since it prevents indiscriminate access of bacteria/products to the portal-venous circulation in healthy conditions. Whereas modulators of this new gut-vascular barrier are yet to be elucidated, known disruptors of the intestinal epithelial barrier are dietary factors (alcohol or overdosed nutrients), mucosal inflammation of any aetiology, drugs/medications (*e.g.* non-steroidal antirheumatics, proton-pump-inhibitors), infections and toxins as well as hypoperfusion[10].

The liver is not only a passive recipient and effector site of gut-derived agents, it also feeds back to the intestine through the secretion of bile, including BAs, as well as other mediators, such as IgA, affecting the gut-liver-axis [11]. Secretory IgAs (sIgA) play a central role in regulating host-microbiota homeostasis [12]. sIgA regulates the composition of the intestinal microflora and protects mucosal surfaces by ensuring immune exclusion. In fact, IgA agglutinates bacteria and participates in biofilm formation, preventing bacterial translocation[13]. Moreover, high IgA coating uniquely identifies colitogenic intestinal bacteria,[14] since transferal

of those bacteria into germ-free mice provided increased susceptibility to colitis. Both intestinal and intrahepatic B-cells produce IgA, most interestingly the latter are derived from Peyer's patches and are directed against intestinal antigens,[15] truly reflecting the liver-gut-axis. The well-known increases in serum IgA levels in chronic liver disease [16] and increased monomeric IgA deposits along the liver sinusoids in alcoholics, even before evolution to overt cirrhosis,[17] underline the highly activated state of the liver-gut-axis in such patients. BAs interact with nuclear receptors and membrane G-protein-coupled receptors, mainly the farnesoid-X-receptor (FXR) and Takeda-G-protein-receptor 5 (TGR5), respectively (Fig.2). Through activation of these receptors BAs regulate lipid, glucose and energy homeostasis in addition to regulating their own synthesis, conjugation and detoxification, as well as modulating intestinal barrier function. Furthermore, BAs modulate the microbiome by exerting antimicrobial actions. Conversely, BA composition is largely determined by the microbiota, since the microbiota modulate the expression of several enzymes involved in BA synthesis and are responsible for biotransformation of primary BA into secondary BA via bile salt hydrolase and 7∝-/β- -dehydroxylation[18]. Some secondary BAs have been reported to increase intestinal permeability and exert pro-carcinogenic action[19]. For instance, an abundance of deoxycholic-acid (DCA) can lead to dysbiosis, which is not only a link in the cancer-progression chain, but has also been connected to obesity and NAFLD/NASH[20].

Once liver cirrhosis has developed, it causes intestinal barrier dysfunction and pathological bacterial translocation that can contribute to complications such as hepatic encephalopathy, spontaneous bacterial infections, hepatorenal syndrome and aggravation of hemodynamic disturbances[21]. Moreover, the BA pool and signalling are vastly altered in cirrhosis.[22] Therefore, liver cirrhosis, acute-on-chronic liver failure and late complications will be discussed separately in each of the following chapters.

Microbiome [1]

This "previously forgotten organ" has vast influence on human health and its role in initiating, perpetuating or aggravating liver disease is beginning to be understood. Multiple independent studies utilising deep sequencing techniques have shown a compositional change and/or dysbiosis in chronic liver diseases,[23] including NAFLD/NASH[24], alcoholism[25,26], cholestatic liver disease[27], cirrhosis[28-30] and HCC[31]. Besides such descriptive observations, quantitative

and qualitative changes in the circulating microbiome (whole blood bacterial DNA) have recently been observed to associate with the development and severity of alcoholic hepatitis, data of which were presented at AASLD[32]. In fact, faecal microbiota transplantation from a patient with severe alcoholic hepatitis into mice renders them more susceptible to alcoholic liver damage, presenting with more severe liver inflammation and necrosis as well as increased bacterial translocation[25]. Similarly, increased small intestinal permeability has been linked to a reduced abundance of *Faecalibacterium prausnitzii* in the terminal ileal mucosa of patients suffering chronic liver disease[33]. However, even more important are changes in microbiota function and their pathophysiological contribution, which needs to be investigated in more detail. Accordingly, an EU-funded project currently aims to define specific gut microbiome and associated host genome, transcriptome and metabolome risk signatures that predict the development and progression of alcoholic liver fibrosis[34]. A recent study demonstrated that successful liver transplantation can restore gut microbial diversity, although it remains impaired relative to controls. Importantly, these changes in gut microbiota are linked with residual or new-onset cognitive dysfunction after transplantation[35]. Thus, therapeutic modulation of the microbiota is a burgeoning area for research. Approaches can be divided into antibiotics, pre-/pro-/synbiotics, dietary changes and faecal microbial transplantation.

Antibiotics [2]

The traditional and most logical first approach to diminish translocation of microbial components and products is to reduce the enteric burden of the bacteria that contribute the most to this, *e.g.* gram-negative bacteria, with antibiotics.

Absorbable antibiotics: Traditionally defined as antibiotics that effectively cross the intestinal barrier to achieve therapeutic serum concentrations, these are used in treatment and prophylaxis of infections. Antibiotics used in liver disease range from norfloxacin and ciprofloxacin for spontaneous bacterial peritonitis (SBP) prophylaxis, to broad-spectrum antibiotic use for those with suspected or confirmed infections. In addition, even pre-emptive efficacy of antibiotic treatment is currently tested in severe alcoholic hepatitis treated with prednisolone[36,37] based on the presumed role of bacterial translocation as a driving force for liver failure in these patients. Moreover, vancomycin has been used in a small but randomised controlled trial (RCT) to treat patients with Primary Sclerosing Cholangitis (PSC), demonstrating a significant improvement in Mayo-Risk-Score, alkaline-phosphatase levels and clinical symptoms[38]. Ongoing trials are investigating the use of vancomycin in the setting of recurrent PSC after liver transplantation[39], as well as in children with PSC, to evaluate the role of gut microflora and its modification[40].

Non-absorbable antibiotics: These are antibiotics which are largely contained within the gut milieu with <4% crossing the intestinal barrier. Traditionally, neomycin, paromomycin and rifaximin have been studied in cirrhosis and hepatic encephalopathy (HE), but of late rifaximin has been recommended owing to its favourable safety profile. Rifaximin, is a broad-spectrum compound, which exerts endotoxin-lowering and anti-inflammatory effects rather than changing the composition of microbiota. *In vitro* studies have shown that this effect is borne out by changes to the functionality of *E.coli* rather than a specific “cidal” activity[41,42]. The form of rifaximin used in clinical practice (550 mg or 200 mg) is hydrophobic and requires BA for its adequate solubilisation and effect. A short-term treatment of 1200 mg/d has been shown to exert beneficial effects in early clinical trials of patients with NAFLD/NASH, lowering endotoxaemia and reducing transaminases [43]. Moreover, rifaximin has proven effective for secondary prophylaxis of overt HE, improvement in cognitive function, health-related quality of life, as well as driving simulator performance in minimal HE[44-46]. The mechanism of the traditional rifaximin compound is like the *in vitro* assay, in that the functionality, *i.e.*

endotoxaemia, is reduced with changes in medium/long-chain fatty acid and bacterial correlations[47]. This lack of major change in microbial composition has been confirmed in larger randomised trials of rifaximin in irritable bowel syndrome and recurrent HE with and without concomitant lactulose use[48,49]. Since patients with advanced cirrhosis, who are the patients most in need of rifaximin, have lower intestinal BA concentrations, the hydrophobicity of the traditional compound could be a hindrance. Therefore, a newer formulation, rifaximin soluble solid dispersion (SSD), which is water-soluble, was introduced. In an animal study, using germ-free mice with and without humanisation from stools from a MHE patient, rifaximin SSD could reduce ammonia production by suppressing intestinal glutaminase activity even in the germ-free state[42]. In the inflammatory milieu induced after humanisation, rifaximin SSD reduced this inflammation as well as the ammonia. A phase II study of rifaximin SSD in patients with early decompensated cirrhosis showed that the 40 mg immediate release formulation could reduce hospitalisations and death, because of reductions in all cirrhosis-related complications, especially HE, in patients with cirrhosis[50]. In view of this, rifaximin could be construed as more than an antibiotic and results from ongoing studies in chronic liver diseases are awaited[51-61]. These trials target different entities (from hepatectomy to NAFLD and cirrhosis) and disease state (non-diseased to decompensated cirrhosis) and aim at hard clinical endpoints (from liver function tests to mortality, see Table 1)

Pre-, Pro-, Synbiotics [2]

In most cases, absorbable antibiotics cause a lasting disruption to the composition of the gut microbiota, which then opens the doors to antibiotic resistance, as well as fungal and pathogen overgrowth (*e.g. Clostridium difficile*) with increased risk of morbidity and mortality*[62]. Therefore, the use of pre-, pro- and/or synbiotics has long been advocated. Currently more than 500 clinical trials are registered at nih.gov., clearly underlining the interest in this field.

Many years ago, pre-biotics, such as inulin, were shown to reduce hepatic lipogenesis and serum triglycerides in humans, [63] attributable to their fermentation by gut microbiota and the associated increase in short chain fatty acids, such as propionate in the colon and portal vein[23]. However, the lack of high-quality clinical trials has recently been emphasised.[64] Ongoing well-designed trials may fill the void and evaluate the effects of prebiotic

supplementation, adjunct to those achieved with diet-induced weight loss, on hepatic injury and liver fat, the gut microbiota, inflammation, glucose tolerance, and satiety in patients with NAFLD[65,66].

A recent meta-analysis of pro/symbiotic use in NAFLD/NASH, utilising different 8 to 30 week regimens, concluded that this approach can produce positive effects[67,68]. Some probiotics lead to a significant reduction in liver transaminases, TNF and insulin-resistance[68]. Such improvements in cytokine profile, insulin action and hence glyceamic control have recently been underlined in another RCT in patients with NAFLD[69]. Additionally, three double-blind randomised placebo-controlled trials were presented at the last AASLD. In a long-term investigation in 39 patients with biopsy-proven NAFLD, VSL3 (12 strains, 675 Billion colony forming units (CFU)/day) was administered for one year significantly improved the NAFLD activity score, with significant improvement in hepatocyte ballooning and hepatic fibrosis[70]. However, only 5/20 patients in the placebo group compared to 10/19 patients in the VSL3 group underwent a second liver biopsy, introducing a risk of selection bias. Nonetheless, VSL3 treated patients presented with reduced serum alanine aminotransferase (ALT), TNF and leptin levels at one year supporting its efficacy[70].

The other two trials aimed at further improvements in NAFLD treatment on top of pre-existing therapeutic interventions, namely sleeve gastrectomy or a very low caloric diet. Bio-25/Subherb (11 strains, 25 Billion bacteria/day) did not significantly impact on hepatic, inflammatory and clinical outcomes, *e.g.* NAFLD remission rate in patients treated for six months after sleeve-gastrectomy[71]. Likewise, probiotic supplementation with *Lactobacillus rhamnosus* and *Bifidobacterium animalis* (14x109 CFU, twice daily) was non-effective in metabolic syndrome post liver transplantation, after four weeks of very low calorie intake (Optifast)[72]. Thirty such patients were randomised to this treatment or placebo for 24 weeks. Probiotics did not significantly alter liver fat content (controlled attenuation parameter [CAP] score), body-mass-index or serum lipids and glycaemia. However, the initial Optifast diet (600 kcal/day) lead to a mean change in total body weight (-6.1 kg), body-mass-index (-2.1 kg/m^2), HbA1c (-3.1 mmol/mol) and CAP score (-24.6 dB/m), in the study population. In summary, evidence for the benefits of pre-/pro- and synbiotics in NAFLD/NASH has accumulated. Hopefully, ongoing large scale RCTs will substantiate current data.[73-77]. However, in the setting of bariatric surgery and/or very low-calorie diet restriction the clinical

impact is less clear.

In alcoholic liver disease, human data on the efficacy of probiotics are scarce but hint towards lowering endotoxemia. A seven day treatment of *Lactobacillus subtilis/Streptococcus faecium* (presumably indicating *Bacillus subtilis* and *Enterococcus faecium*[78]) in alcoholics undergoing strict abstinence, during the same observation period, reduced serum endotoxin levels[79]. The same authors are currently investigating *Lactobacillus rhamnosus* R0011 and *acidophilus* R0052 (Lacidofil) in a placebo-controlled trial, which aims to improve liver enzymes in alcoholic hepatitis[80]. In patients PSC, probiotics (*Lactobacillus/Bifidobacillus*) have only been tested in one small pilot trial so far, with no significant changes in liver biochemistry at the end of three months treatment[81,82]. In liver cirrhosis, utilisation of pro-/synbiotics has traditionally been tested for its impact on HE. In fact, a recent meta-analysis of 14 studies, including a total of 1,152 patients with cirrhosis, demonstrated that probiotics are effective at improving minimal HE (MHE) and preventing progression to overt HE, as well as reducing hospitalisation rate in patients with underlying MHE[83]. As for secondary prophylaxis, probiotics demonstrated non-inferiority to lactulose in one trial,[84] but symbiotic treatment using VSL3 failed[85]. However, treatment with VSL3 for six months, in patients who had recovered from an episode of HE during the previous month, clearly reduced the rate of hospitalisation for HE or other complications of cirrhosis with an impressive number needed to treat (NNT) = five, to avoid one hospitalisation in six months. In fact, this efficacy is numerically equivalent to that reported for rifaximin plus lactulose[44]. In addition, VSL3 improved the quality of life and decreased both Child-Turcotte-Pugh-class and Model of End-Stage Liver Disease (MELD) scores [85]. This further supports prior data pointing to improvements in liver function by pro-/symbiotic strategies in cirrhosis [86,87]. In terms of haemodynamic improvements VSL3-RCTs revealed a small trend to lower hepatic venous pressure gradient (HVPG) in compensated and early decompensated patients,[88] and a significantly improved HVPG-response additive to propranolol in advanced cirrhosis[89]. However, with probiotics, the CFUs present in commercially available formulations may not be consistent, since they are marketed as dietary supplements in most countries. Therefore, only pharmaceutical grade probiotics, which have been used in the trials above, should be used in patient care.

Finally, in the setting of liver transplantation, a meta-analysis of four controlled studies demonstrated that giving patients a combination of pre- and probiotics before or on the day of

transplantation reduces the rate of infections after surgery and shortens hospital and intensive care unit (ICU) stays, as well as antibiotic usage[90]. An additional placebo-controlled trial using a four-strain probiotic from enrollment till liver transplantation could demonstrate significantly reduced 30- and 90-day infection rates, lower post-liver transplant bilirubin concentration and more rapid decrease in transaminases, but no change in 90-day mortality (0% *vs.* 4.3%)[{Grat, 2017 #842}]. In terms of symbiotics, ongoing RCTs also assess post-liver transplant infection rates,[92] as well as its effect on morbidity in patients with liver fibrosis (F3/4) undergoing liver resection for HCC.[93]

Taken together, evidence for the potential clinical benefits achievable with pre-/pro-/synbiotics in chronic liver diseases including NAFLD/NASH, alcoholic liver disease and liver cirrhosis accumulates. It should be underlined that no severe adverse effects have been reported in RCTs using pre-/pro-/synbiotics in this setting, which should broaden its application. However, the beneficial effects induced by pre-, pro-, synbiotics are largely individual, owing to vast differences in diet, host genetic background and micromilieu in the gut. This heterogeneity makes effects unpredictable in extent and duration. Hence, the next challenge will be to find factors that predict good responses and treatment individualisation.

Faecal microbial transplantation (FMT) [2]

Logically, transplantation should restore a “healthy” microbiome. FMT has not been observed to increase rates of adverse events in severe alcoholic hepatitis, NASH and cirrhosis, in clinical practice or published data.[82,94-97] However, these data need to be interpreted cautiously, since the included cirrhotic patients either had MELD< 18 or presented with a mean Child-Pugh-Score of 8[97]. In fact, only one decompensated patient with a Child-Pugh-Score >10 underwent FMT for refractory *clostridium difficile* infection and did not achieve a response[97]. Therefore, these promising data need confirming in larger cohorts of patients with various aetiologies and advanced stages of cirrhosis, with long-term follow-up.

In terms of efficacy, a well-designed RCT on FMT via rectal enema is available utilising stool from one ideal OpenBiome donor that has been selected using cross-sectional HE microbiome data and being applied after a five-day course of antibiotic pre-treatment in

patients with cirrhosis and≥2 HE episodes on lactulose ± rifaximin. During a median follow-up of 100 days FMT, but not standard of care, reduced hospitalisations (10 *vs.* 1) and improved cognitive function, evaluated by PHES and Stroop App. FMT increased beneficial taxa (Bifidobacteriaceae, Lactobacillaceae), accompanied by a favourable change in urine metabolomics with reduced microbial products, such as hippurate and phenylacetylglycine. Interestingly, antibiotic pre-treatment worsened MELD score and reduced autochthonous taxa with expansion of proteobacteria. FMT reverted MELD score to baseline and restored antibiotic-associated microbial changes. Although the number of patients who have undergone FMT is still low, these data outline potential benefits [98]. FMT was compared with pentoxifylline (400 mg Q8H for 28 days), in an Indian RCT, where it was given via daily jejunal tube application, for seven days, in severe and steroid-refractory alcoholic hepatitis. FMT resulted in lower rates of complications (HE, ascites, renal dysfunction) with concomitant improvements in ammonia, TNF and bilirubin. It ultimately enhanced survival at three months compared to pentoxifylline[96]. Although not further detailed, the selection process with 121/151 patients excluded could indicate that there is a risk of selection bias. Hence, these data could hint towards a particularly potent effect of FMT in the most severe course of acute alcoholic hepatitis.As for the changes in the microbiome induced by FMT. Increased *Bacteroides* and decreased pathogenic *Proteobacteria* have been demonstrated but recipient microbiota showed only distinct community variation post-FMT in severe alcoholic hepatitis [96]. Thus, the future needs to focus not only on “who” is transplanted and colonises (or not) but also on the transplanted microbial functionality. Although the methodology has improved and could deliver valuable knowledge, regulatory issues are increasingly cumbersome and create obstacles in performing such FMT clinical trials. Nonetheless, the huge interest in FMT and its great potential in liver disease is reflected in numerous ongoing trials in the transplant setting (for treatment of multi-drug-resistant organisms[99]), liver cirrhosis (feasibility[100] and rate of complications[101]), NAFLD/metabolic syndrome[102,103] as well as PSC[104]. Finally, more basic information as to whether pre-conditioning the patient/colon with bowel preparation and/or antibiotics is helpful and/or necessary, needs to be defined.

Adsorbents [2]

A completely different approach involves using poorly absorbable, adsorptive material to bind gut-derived toxins and bacterial products, thus abrogating their inflow into the liver and systemic circulation. This material can then be excreted in the stool. In fact, ion exchange resins targeting hyperkalaemia and hyperphosphataemia have shown efficacy, providing proof of concept for adsorption as a therapeutic strategy[105]. Cholestyramine is effective as a treatment for pruritus by binding BA in the gut lumen [106]. Lactulose, a non-absorbable disaccharide effectively reduces ammonia absorption in the gut and is an effective treatment for HE.

Recent advances in activated carbon technology have led to the development of synthetic adsorptive nanoporous carbons. They have uniquely tailored porosity that is acquired during synthesis and activation. AST-120 (Kureha Corporation, Japan), a carbon bead that acts as a device in the gut lumen and can bind substances in the microporous range is used widely in patients with chronic kidney disease, particularly in Japan[107]. Studies using AST-120 in bile duct ligated rats showed that it effectively reduced ammonia concentration, resulting in a reduction in brain water. This has therapeutic potential in patients with hepatic encephalopathy[108]. However, a large study in patients with minimal HE did not demonstrate a clinical benefit, partly owing to a high learning effect of the testing strategy used. Further development of AST-120 for this indication has been halted[109]. To overcome the limitations of AST-120, a novel synthetic activated carbon, Yaq-001 (Yaqrit Ltd. UK), with tailored porosity in both the microporous and macroporous range was developed (Fig.3). Yaq-001 is excreted unchanged in the stool. It is regulated as a device in Europe and its oral administration could reduce the trans-intestinal migration of bacterial endotoxins produced by organisms resident in the gut[110]. *In vitro* studies have shown that Yaq-001 preferentially removes hydrophobic substances and those with molecular weights up to approximately 70kDa. Therefore, Yaq-001 can specifically adsorb gut-derived toxins such as ammonia, asymmetric dimethylarginine, acetaldehyde, hydrophobic BA, cytokines such as TNF alpha and interleukin-6, as well as bacteria derived products like LPS and exotoxins (Fig. 3). Proof-of-concept studies administering Yaq-001 to bile duct ligated rats, confirmed its impact on the removal of these molecules and results in pleiotropic beneficial effects, characterised by a reduction in the severity of endotoxaemia, liver injury, markers of systemic and hepatic inflammation, portal hypertension, renal dysfunction and severity of hepatic

encephalopathy[110]. It is important to note that this effect is not associated with any antibacterial properties *in vitro,* but changes in the microbiome of the bile duct ligated rats was observed when administered *in vivo*[111]. Although the significance of these changes is not clear, the data indicated that alterations to the micro-environment, induced by Yaq-001, change the gut microbial flora[112]. Preliminary studies in rodents with NAFLD show a marked reduction in steatosis and hepatic inflammation[112]. These early observations provide the first indications of the potential of this non-antibiotic, adsorptive strategy. Clinical trials of Yaq-001 are being implemented as a part of the European Commission Horizon 2020 programme (carbalive.eu). As Yaq-001 is regulated as a device in Europe, a safety and performance study is being performed, which should lead to a CE-mark (CARBALIVE-SAFETY)[113]. Two further efficacy studies are then planned. The first is in patients with cirrhosis, and acute decompensation (PREVENT-ACLF). The main endpoint of this study looks to determine whether the administration of Yaq-001 prevents the recurrence of cirrhosis-related complications and the associated hospitalisation. The second efficacy study is planned in patients with NAFLD (TREAT-NAFLD). As Yaq-001 is a non-specific adsorbent, novel delivery methods are being developed to prevent potentially deleterious effects resulting from binding of essential nutrients and drugs.

Bile acids [1]

BA can be considered the common language of communication along the liver-gut-axis. The interplay between BA and microbiota in the gut changes BA composition and influences BA homeostasis and different metabolic processes in the host, through FXR and TGR5 receptor signalling [114,115]. The nuclear receptor FXR, the major regulator of BA homeostasis, is expressed in hepatocytes, ileal enterocytes and kidneys.Its most potent ligands are the primary BA, chenodeoxycholic acid (CDCA) and cholic acid (CA). Activation of FXR in ileal enterocytes induces the expression of the enterokine fibroblast growth factor (FGF)15 (in mice)/19 (in humans), which reaches the liver through the portal vein, activates the FGFR4/b-klotho complex on hepatocytes, and inhibits the expression of CYP7A1, the rate limiting enzyme in the classical pathway of BA synthesis (Fig. 2). In hepatocytes, binding of BA to FXR induces a small heterodimer partner (SHP) that also downregulates CYP7A1, decreasing BA synthesis. Recent evidence also indicates that ileal FXR activation leads to

stronger suppression of CYP7A1-mediated BA synthesis than hepatic FXR activation[116]. Therefore, the gene expression program activated by FXR reduces intracellular BA levels by increasing the transport of BA out of cells, decreasing BA uptake and lowering BA synthesis. Activation of enterocyte or hepatocyte FXR also has several downstream metabolic effects, including increased glycogen synthesis, reduced gluconeogenesis and increased free fatty acid oxidation. FXR activation on hepatocytes also leads to inhibition of lipogenesis and increased insulin sensitivity, decreasing hepatic steatosis. Notably the FXR-agonist obeticholic acid (OCA, 6α -ethyl-chenodeoxycholic acid) decreased intestinal glucose absorption in healthy volunteers[117] although the mechanisms are still unclear.

Considering the marked effects of FXR activation on glucose and lipid metabolism, it is conceivable that molecules that modulate these receptors (*i.e.* FXR-agonists) or levels of endogenous BA (*i.e.* BA transporters) might have beneficial effects on NAFLD/NASH. The most clinically developed FXR-agonist is OCA (Intercept Pharmaceuticals, New York, USA), a steroidal semisynthetic BA-derivative, which has been used in two published trials, one in PBC[118] and one in NASH (The FLINT trial)[119]. In a double-blind, placebo-controlled trial, 183 patients with NASH without cirrhosis were randomised to receive either 25 mg of OCA or placebo for 72 weeks. The study was stopped early, since OCA significantly improved the primary histological outcome (*i.e.* NAFLD activity score) and reduced liver fibrosis compared with placebo. However, the FLINT trial raised several safety issues, including i) pruritus in about 20% of OCA-treated patients, ii) dyslipidaemia, with increased total- and low-density lipoprotein (LDL)-cholesterol and mild, but significant, reductions in high-density lipoprotein (HDL)-cholesterol, and iii) concerns regarding the carcinogenic potential of increased circulating FGF19, since overexpression of FGF19 has been associated with HCC in animal models [120]. A phase III trial evaluating the effect of OCA on mortality and liver-related outcomes, including HCC at five years, is currently recruiting patients with non-cirrhotic NASH[121]. Additionally, the efficacy of OCA in alcoholic hepatitis and PSC is currently being investigated in two phase II double-blind placebo-controlled trials[122,123].

The results of the FLINT trial have encouraged the search for more efficient and safer FXR-agonists than OCA. FXR-agonists have been developed with two basic structures: steroidal semisynthetic ligands and non-steroidal fully synthetic ligands. A proof-of-concept phase II study with the non-steroidal FXR-agonist, PX104, in patients with NAFLD showed the

relevance of these compounds for the treatment of metabolic liver disease (Phenex Pharmaceuticals AG, Heidelberg, Germany)[124]. The authors observed that a four-week course of PX-104 improved insulin sensitivity (evaluated by clamp like index) and decreased serum gamma-glutamyltransferase (GGT) and ALT in 12 non-diabetic patients with NAFLD, without changes in serum lipids and FGF19. However, treatment had no impact on hepatic steatosis (evaluated ^{1}H-MRS and PDFF-MRI), probably because of low-dosage and the short study period. The study was prematurely interrupted because of intervals of cardiac arrhythmia in two patients.

Gilead Sciences (USA) has developed GS9674, the follow-up compound to PX-104, which has an improved efficacy and safety profile. In contrast to OCA, GS9674 and similar agents are less likely to undergo enterohepatic circulation, which confers more predictable pharmacokinetics and gut-preferential activity. GS9674 reduces hepatic steatosis, increases FGF19, reduces serum BA, and reverses dyslipidaemia, without changing HDL levels in preclinical models of diet-induced obesity, providing support for its investigation in patients with NASH[125-127]. The preclinical data have been confirmed in a randomised placebo-controlled study in healthy volunteers receiving different oral doses of GS9674 for two weeks[128-130]. In this study, oral GS9674 increased FGF19, reduced serum BA, without changing serum lipid levels. Administering GS9674 with food lowered systemic exposure[128,129]. Ongoing trials will address the safety and efficacy of GS9674 alone in patients with NASH,[131] or combined with selonsertib in patients with steatosis and elevated liver stiffness[132]. Selonsertib (formerly GS4997) is an inhibitor of the apoptosis signal-regulation kinase that, alone or in combination with the monoclonal antibody simtuzumab, has been shown to reduce liver fibrosis and liver fat content in patients with NASH and moderate to severe liver fibrosis (stages F2 and F3)[133]. Finally, Intercept Pharmaceuticals is about to launch a phase I trial with INT-767, a BA analogue that acts as a dual agonist on FXR and on TGR5. In mice models of NASH, INT-767 improves plasma and hepatic lipid profiles, and reduces systemic and hepatic inflammation[134-136]. The functions of TGR5 extend beyond metabolic regulation, and include modulation of the inflammatory response, peristalsis and liver regeneration[137]. However, individual TGR5-focused trial programmes have been hampered by identified risks (*e.g.* gallbladder distension), adverse effects and questions about the translation of animal data to humans[138].

LJN452 (Novartis, Basel, Switzerland) is another non-BA FXR-agonist currently entering a phase II trial to assess efficacy, safety and tolerability in patients with NASH[139]. The first in human experience with LJN452 was recently described. A two-week course of single and multiple ascending doses of LJN452 in healthy volunteers was found to be safe and to cause a transient dose-dependent increase in FGF-19, a marker of intestinal FXR engagement, without increasing LDL or cholesterol or eliciting itch[140]. A different approach to modulate BA signalling could be to block ileum reabsorption of BA, favouring its excretion in faeces and forcing the liver to synthesise new BA from cholesterol in the liver and serum. Palmer *et al.* presented the results of a phase I placebo-controlled study of a 12-day course of volixibat, a minimally absorbed, oral, inhibitor of the apical sodium-dependent BA transporter (ASBT), in 84 overweight/obese subjects[141,142]. Volixibat increased daily faecal BA excretion in a dose-dependent manner, reduced total and LDL-cholesterol and increased bowel movements, providing support for the ongoing trial of this compound in patients with NASH [143]. One way to directly substitute primary BA orally is Aramchol, a novel synthetic small molecule produced by conjugating two natural compounds, the fatty acid arachidic acid, and the BA, CA, by a stable amide bond. Aramchol reduces liver fat by partial inhibition of the liver stearoyl-CoA desaturase-1, an enzyme which leads to hepatic insulin resistance and reduces neoglucogenesis, and ABCA1 transporter-mediated cholesterol efflux stimulation. Aramchol has been tested in a small phase 2 study in biopsy-proven NAFLD patients, where it reduced liver fat, as measured by MRS, without any safety concern[144]. There is an ongoing phase IIb clinical trial in patients with histologically proven NASH, high liver fat content and features of metabolic syndrome, where reduction in liver fat by MRS is the main endpoint[145]. Finally, microbial biotransformation of bile salts via deconjugation and dehydroxylation can greatly affect their FXR agonistic properties. Probiotics (*i.e.* VSL#3) have recently been shown to modulate the enterohepatic FXR/Fgf15/CYP7A1 axis. Enrichment of microbiota in mice with the bile salt hydrolase retaining *Lactobacilli* and *Bifidobacteria* promotes BA deconjugation and faecal excretion, repression of the enterohepatic FXR/Fgf15 axis, increased hepatic BA neosynthesis and negative BA reuptake[146]. This approach could be relevant in pathological conditions such as bacterial translocation, secondary to intrahepatic cholestasis or inflammatory colitis.

Available preclinical and clinical data indicate that modulators of the FXR pathway are promising for the treatment of metabolic liver disease. However, research in this area faces several challenges, including safety issues and selectivity of targeting at the level of hepatocytes, intestine or other sites. A recent study using an intestinal-restricted FXR agonist, fexaramine, resulted in activation of FXR in the ileum, which was associated with less weight gain, lower insulin resistance, and decreased hepatic steatosis in mice on a high-fat-diet[147]. These data point to the intestine as the optimal target tissue to treat liver disease. In fact, OCA has been shown to reduce bacterial translocation and stabilise the intestinal barrier, and is associated with a reduction in liver fibrosis in experimental cirrhosis[148-150]. These data are in accordance with the observation that FXR-agonists augment synthesis of intestinal antimicrobial peptides and lectins[150,151] and significantly decrease TNF-secretion in purified CD14 monocytes and dendritic cells, as well as in lamina propria mononuclear cells from patients with irritable bowel disease[152]. Finally, BA stimulates intestinal enterochromaffine cells to secrete 5HT/serotonin hence positively regulating persistalsis. Taken together, these findings underline the beneficial action of FXR-agonists on the intestinal barrier and warrant further investigation in human advanced fibrosis and cirrhosis.

Gut-derived Hormones [1]

Gut-derived hormones play a key role in controlling energy intake and homeostasis, since they are released in response to nutrients. Thus, they provide the opportunity for rapid metabolic feedback loops. In addition to nutrients, microbiobial products and metabolites modify hormone release. Incretins such as glucagon-like peptide-1 (GLP-1) augment glucose-mediated insulin secretion, inhibit glucagon release, slow nutrient absorption by reducing gastric emptying and reduce food intake, making them attractive therapeutic targets for NAFLD/NASH. The GLP-1 analogue, liraglutide, reduced NAFLD activity score and fibrosis stage and improved metabolic parameters in a diet-induced obese mouse model of NASH[153]. Notably, liraglutide de-activates hepatic stellate cells (HSC) and exerts antifibrotic effects, and it improves sinusoidal microvascular dysfunction in rats and precision-cut human liver slices. However, HSCs do not appear to express the GLP-1 receptor, pointing towards potentially important off-target effects{de Mesquita, 2017 #843}, which may contribute to the effects observed in

recent clinical studies. Liraglutide has recently been shown to be safe, well tolerated, and led to histological resolution of NASH, although the anti-fibrotic effects in this small study were rather disappointing[155]. Similar studies have explored the effects of exenatide in NASH[156,157]. An ongoing study is comparing the effects of liraglutide and bariatric surgery on weight loss, liver function, body composition, insulin resistance, endothelial (dys)function and biomarkers of NASH in obese Asian adults[158]. In addition, a range of more mechanistically oriented studies currently explore and compare the effects of various incretin minimetics on hepatic lipid and lipoprotein metabolism[159-161]. Interestingly, the clinical results obtained with dipeptidyl peptidase-4 inhibitors such as sitagliptin in NAFLD/NASH, have been somewhat disappointing[162,163]: Sitagliptin was safe but no better than placebo at reducing liver fat in prediabetic or diabetic patients with NAFLD. Interestingly BA also stimulated the release of GLP-1 via TGR5 on intestinal L-cells. In fact, stimulation of local GLP-1 release by BA or TGR-5 analogues has been suggested to be superior to exogenous GLP-1 receptor agonists for controlling type 2 diabetes and obesity[164].

Progression of NAFLD to NASH and more advanced fibrosis has always been tightly linked to the severity of metabolic dysregulation. Interestingly, postprandial hyperglycaemia and hyperinsulinaemia are associated with advanced fibrosis in patients with diabetes and NASH[163]. This raises the critical question whether direct control of hyperglycemia may also have a positive impact on fibrosis progression of NASH. Sodium-dependent-glucose-transporter (SGLT)-2 inhibitors are newly FDA-approved oral medications used to treat type 2 diabetes, and have been shown to reduce production and deposition of fat in the liver in animal experiments. In a small Japanese pilot trial, an SGLT-2 inhibitor (ipragliflozin) improved hepatic inflammation and fibrosis of NAFLD patients with type 2 diabetes{Ohki, 2016 #844}. Two RCTs with Empagliflozin, another SGLT-2 inhibitor, in patients with type 2 diabetes and NAFLD are currently ongoing[166,167].

As outlined before BA/FXR-mediated release of the enterokine FGF15 (in mice)/19 (in humans) in ileal enterocytes plays a key role in the regulation of BA synthesis, lipid and glycogen metabolism in the liver (recently reviewed[168]). Hence, FGF19 mimetics have been engineered but exert potentially carcinogenic effects, since FGF19 induces hepatocyte proliferation through FGFR4 receptor activation. Thus, an important concern may exist in precancerous conditions such as NASH, since overexpression/amplification of FGF19 or its

receptor FGF4R has been observed in HCC and contributes to progression and resistance of HCC[169]. In contrast, NGM-282 is a recombinant variant of FGF-19 that was found to retain the metabolic, but not the tumourigenic effect of FGF-19 in preclinical models. Indeed, preliminary reports from a recent study demonstrated that human FGF19, but not NGM282, caused HCC in a diet-induced mouse model of NASH[170]. NGM282 has been successfully tested in phase II studies in primary biliary cholangitis (PBC), demonstrating effective suppression of BA synthesis and improvement of cholestasis.[171] Studies in PSC[172] and NASH[173]are still ongoing.

Another member of the “endocrine” FGF subfamily is FGF21, which is released from the liver in a PPAR-alpha regulated fashion. FGF21 stimulates glucose uptake, browning of adipocytes and energy expenditure in rodents. Thereby, FGF21 protects them from diet-induced obesity and diabetes, and represents an attractive therapeutic target in NAFLD/NASH. The first data obtained with pegylated recombinant forms of human FGF21 (BMS-986036) in preclinical mouse models of NASH[174], phase I studies in healthy volunteers [175] and obese subjects [176], as well phase II results obtained in obese adults with type 2 diabetes and presumed NAFLD[177] are encouraging. However, meaningful hepatological endpoints have not yet been reported and a phase II study in NASH is ongoing[178]. Some groups are taking this strategy even further by testing long-acting GLP-1/FGF21 dual agonists. As such, preliminary data have demonstrated improvement of hepatic steatosis/repression of lipogenesis, inflammation and fibrosis with YH25724 (a novel long-acting dual agonist, which is an immunoglobulin Fc-fused protein comprising a GLP-1 variant and an FGF21 variant) in three mouse models of NASH[179]. The fusion strategy can also be applied to other chimera: MEDIO382, a dual GLP-1/glucagon receptor agonist has beneficial effects on mitochondrial content and function in primary hepatocytes from lean and NASH mice,[180] and improved metabolic and hepatic indices of NASH in mice [181]. In view of this, multiple drugs implementing the enterokine pathways are in the pipeline;. preclinical and early clinical experiences are promising in metabolic liver diseases.

Nerves, Peristalsis and beyond [1]

Since the gut and the liver are densely innervated by parasympathetic and sympathetic nerves this also represents a potential target. Intestinal sympathetic hyperactivity, with increased release of norepinephrine is present in liver cirrhosis[182,183]. Non-selective beta blockers (NSBBs) are the fundamental pharmacological approach for prevention of variceal haemorrhage[184]. NSBBs ameliorate pathological bacterial translocation in experimental cirrhosis[185], most likely by reducing intestinal permeability[186], improving intestinal transit time, inhibiting bacterial overgrowth and improving cellular host defense in the gut-associated lymphatic tissue[187]. In fact, NSBBs are associated with reduced severity of systemic inflammation and improved survival of patients with acute-on-chronic liver failure[188]. They have been proposed to reduce the risk of HCC by lowering the M/PAMP-aemia in the portal circulation[189]. Although sympathetic nervous system catecholamines are upregulated in human NAFLD[190] and amplify experimental LPS-induced liver injury[191], no trials on the potential effect of beta-blockers in alcoholic or non-alcoholic fatty liver disease are available.

Intestinal *vagal nerves* are involved in regulating nutrient absorption, food intake, glucose homeostasis and body weight[192]. Moreover, cholinergic anti-inflammatory action[193] is well-accepted and should also guide treatmentin liver diseases. Experimentally, vagal nerve stimulation has been demonstrated to lower portal pressure[194]. New tools for vagal nerve stimulation are now available and are being actively evaluated to substantiate their anti-inflammatory potential in healthy individuals[195], as well as in chronic inflammatory bowel disease [196]and to counteract obesity[197] creating a novel strategy in chronic liver disease.

Long-term treatment with the prokinetic 5-HT4-agonist cisaprid, in patients with liver cirrhosis, reversed small intestinal dysmotility and bacterial overgrowth. It also improved liver function[198], most likely via weeping the gut, thereby lowering pathological bacterial translocation[199]. However, cisapride has been abandoned because of its adverse cardiac effects. The new generation 5-HT4-agonist masopride appears safe and besides accelerating gastro-duodenal transit, it also exerts anti-inflammatory effects [200]and promotes neurogenesis[201]. This warrants its assessment in cirrhosis, with the aim of reducing pathological bacterial translocation.

The majority of mammalian *serotonin* is synthesised from the essential amino acid tryptophan in intestinal enterochromaffine cells of the gastrointestinal tract. This synthesis is regulated by metabolites derived from the microbiota, particularly spore-forming bacteria[202], once more underscoring the fundamental role of the microbiome in regulating gut function. Serotonin is involved in a variety of pathological conditions of the liver, including chronic cholestasis[203], NASH[204], liver fibrosis[205] and regeneration[206,207]. The beneficial effect of 5-HT in promoting liver regeneration is particularly attractive considering that reduced preoperative intraplatelet 5-HT is associated with an increased incidence of postoperative liver dysfunction and morbidity and poor clinical outcome[207]. An ongoing observational trial will delineate the role of platelet-derived factors, *e.g.* 5-HT/serotonin, in liver regeneration after liver resection in more detail[208]. Conversely,serotonin may facilitate growth of HCC, since 5 HT has been shown to promote proliferation in hepatic cancer cells and human HCC tumours [209,210]. In fact, a recent investigation in patients undergoing liver resection links increased intraplatelet 5HT-levels prior to surgery to early tumour recurrence[211]. Thus, serotonin is a double-edged sword for the liver. More translational data are necessary to unravel the role of gut-derived serotonin and its specific intrahepatic action before therapeutic trials can take advantage of this information.

Bariatric Procedures [1]

The strong causal association between obesity and NAFLD implicates the potential benefits of bariatric measures on the liver.Since bariatric procedures usually achieve weight loss greater than 10% after one year, which is associated with metabolic improvements and is almost unobtainable by lifestyle interventions alone, indications for bariatric surgery are clear[212]. However, bariatric surgery is not currently recommended for NAFLD/NASH, since mortality in those patients is dictated by cardiovascular events[213]. Nonetheless, it is accepted that gastric bypass can affect the gut microbiota favourably by lowering the proportion of firmicutes[214], remodelling the bile acid pool and modulating secretions of incretins (GLP-1/-2)[215]. Hence, this affects all the previously outlined pathways usedto target the liver-gut-axis.

In fact, gastric bypass has been shown to induce the disappearance of NASH in nearly 85% of patients (particularly in those with mild disease) and to reduce fibrosis in stages F1/2

[216]. Although this trial has been criticised for being a single centre study, without a control group and rates of NASH that are too low,[217,218] it outlines the potential benefits. According to guidelines for adolescents, bariatric interventions are recommended even in children with severe obesity, with NASH and significant fibrosis (>F1), when other treatments have failed.Additionally, the use of sleeve gastrectomy in a prospective pilot trial in obese adolescents with NASH has just been published{Manco, 2017 #845}. Sleeve gastrectomy was more effective than lifestyle interventions for reducing NASH and liver fibrosis after one year of treatment. Ongoing randomised trials compare gastric bypass and sleeve-gastrectomy[220] and will help to establish their role in NAFLD with type 2 diabetes [221]. Until then, at least severely or morbidly obese patients (BMI 40 or BMI 35 to 40 with other comorbidities) with NAFLD/NASH , should be offered bariatric surgery unless advanced fibrosis is present.

Endoscopic approaches are developed to substitute for bariatric surgery. At the AASLD 2016, intragastric balloon placement was reported to not only lower body weight and BMI (mean reduction 4.1), with improvements in homeostatic model assessment of insulin resistance (HOMA-IR), but also to significantly improveme liver transaminase levels in morbidly obese patients (mean BMI 42 + 6)[222]. Non-endoscopic insertion of gastric balloons, by swallowing and inflating, is also available, which could easily expedite this approach. A device mimicking gastric bypass by shielding the duodenum and upper jejunum from contact with the chyme is inserted and anchored endoscopically (Endobarriere). Its use in severely obese patients (mean BMI 38+9), with NAFLD and type 2 diabetes, decreased BMI, waist circumference, HbA1c and lipid profile after six months [223]. Moreover, liver fibrosis stage, liver fat content and steatohepatitis improved significantly, as evaluated by shear-wave elastography, steatotest and Nashtest/Fibromax.

A more targeted approach without implantation of any device is hydrothermal duodenal mucosal resurfacing. The proximal duodenal mucosa is denuded before mucosal restitution of “healthy” neo-epithelium. First data in abstract form on 52 patients with moderate obesity and type 2 diabetes revealed a significant drop in HbA1c of about 1.0 % at six months and a significant 25% decrease in transaminases, sustained at 12 months follow-up[224]. This method does not depend on implantation of any device and reflects more direct interference with pathophysiological relevant gut-hormone derangement and restoration.

Conclusions and outlook [1]

It is well recognised that the gut-liver axis plays an important role in the etiology of many liver diseases. Consequently, it is logical to seek to manipulate this axis. Indeed, the last years have turned the page from basic and animal research to clinical trials, aiming to translate this knowledge into therapeutic and/or preventative measures, in human liver diseases. The best example is the developmental pipeline of pharmacological approaches mimicking *bile acid* or enterokine signalling *e.g.* via FXR-agonists or GLP1-agonists. Based on the well-accepted role of BA in intestinal barrier function, as well as glucose and lipid metabolism, pharmaceutical companies have realised the outstanding hepatological and systemic health benefit that this class of drugs can provide,. Initial clinical trials, mainly phase I and individual phase II investigations, underline the prospect of this approach in lowering liver fat, inflammation and fibrosis in metabolic liver disease. We feel strongly that the gut is the optimal primary target and that FXR-agonists that do not undergo enterohepatic circulation in terms of pharmacokinetics, such as intestinal-restricted FXR-agonists are particularly promising. Likewise, recombinant enterokines have shown encouraging results in metabolic liver diseases. Despite these, somewhat foreseeable, beneficial metabolic effects, large scale phase II/III clinical trials with hard clinical endpoints and long-term follow-up are needed. These investigations are underway and will clarify the effect size and particularly safety issues. In fact, detailed knowledge of FXR-signalling in different tissues and reproducible pharmacological modulation should enable the generation of high-quality clinical data for all groups of patients with liver disease.

In contrast, the microbiome is highly individual. It is exclusively complex and intensely difficult to modulate in any predictable or sustainable way, thus hampering performance of clinical trials. Nonetheless, the microbiome can be considered the “core facility“ for the production of a myriade of bacterial metabolites and products to which the gut-vascular barrier and each member of the gut-liver-axis are exposed. Traditional antibiotics seem unlikely to be an effective means of promoting a helpful host-microbiota relationship, and are ultimately hampered by adverse effects and the emergence of antibiotic resistant bacteria. Pre-/pro- and synbiotics however, can be considered safe and evidence of their positive effects in alcoholic, non-alcoholic liver disease, cirrhosis and liver transplantation is accumulating. Considering the experimental evidence that probiotics, besides multiple beneficial effects on the intestinal

barrier and immune function, are also modulating FXR-signalling further strengthens the rationale behindthis treatment strategy. The efficacy and safety of FM is less well defined. Nonetheless, the potential effects and mechanisms are starting to be understood, which should lead to a more targeted approach.

In times of almost epidemic rates of obesity and associated liver diseases, hepatology needs to consider bariatric measures within its armamentarium.For instance, non-surgical techniques delivered by endoscopy, such as duodenal mucosal resurfacing, could substitute for surgery. However, so far only early clinical data in small numbers of patients, with various disease degree and severity, are available. It will be fascinating to see the results of future clinical trials.

Considering the various treatment concepts available, such as adsorbents (Yaq-001), utilisation of vagal nerve stimulators and new prokinetic 5-HT4-agonists the armamentarium to target the gut-liver-axis will continue to expand. We hope that you share our gut-feeling that the preventative and therapeutic strategies, translated from our current knowledge of the liver-gut-axis, provide an exciting future in liver treatment, which will benefit our patients.

Financial support [1]

The authors acknowledge the CARBALIVE team, in particular Susan Sandeman, Ganesh Ingavle and Jane Macnaughtan for the Yaq-001 data. The CARBALIVE project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634579. RW is funded by Swiss National Fund (SNF 17-231)

Conflict of Interest [1]

Professor Jalan is an inventor of Yaq-001, the patent of which has been licensed into a UCL spinout company, Yaqrit Ltd. Professor Jalan is one of the founders of Yaqrit Ltd. MT serves as a consultant for Albireo, Falk, Genfit, Gilead, Intercept, MSD, Novartis and Phenex and is a member of the speakers' bureau of Falk, Gilead, MSD and Roche. He further received travel grants from Falk, Roche and Gilead and unrestricted research grants from Albireo, Falk, Intercept and MSD. He is also co-inventor of a patent on the medical use of nor-UDCA. JSB has served as a consultant for Salix, Norgine, Grifols and Abbott Pharmaceuticals. His institution has been funded for research trials from Salix and Grifols

Authors' contributions [1]

RW, JB, AA, MT, RJ contributed to this manuscript by selecting and summarising the information delivered at the Liver Meeting held in San Francisco in November 2016 and selecting ongoing trials in their respective fields of expertise.

Table 1: Ongoing Clinical Trials targeting the Liver-Gut-Axis

CP, Child-Pugh-Score; FMT, faecal microbial transplantation; FU, follow up; HOMA, Homeostasis model assessment of insulin resistance; HVPG, hepatic-venous-pressure-gradient; MD, Maddrey discriminative score; SSD, soluble solid dispersion; T2D, Typ-II-diabetes; D, day; W, week; Mo, month; Y, year; the table only considers ongoing, recruiting trials with an update available at least within one year at www.clinicaltrials.nih.gov. For bariatric trials see text. Trial phase for pre-/pro-/synbiotics is mostly not presented hence not stated.

Figure legends

Fig. 1: Pathophysiology of Gut-Liver-Axis:

The microbiome sets the stage for the gut-liver-axis, representing an excessive source of bacterial products and metabolites in terms of both quantity and diversity. In conditions of increased intestinal permeability, the epithelial barrier is crossed more than in healthy conditions by bacterial products (lipopolysaccharides, peptidoglycans, bacterial DNA, flagellin etc.), which stimulate the gut-associated lymphatic tissue to release pro-inflammatory cytokines (TNF, IL1, IL6 etc.), chemokines, as well as eicosanoids, leading to portal-venous M/PAMP- and cytokinemia. Moreover, bacterial metabolites (trimethylamine, ethanol and other volatile organoids, fatty acids, acetaldehyde etc.) increasingly permeate the epithelial barrier. Anything crossing the epithelial barrier faces the gut-vascular barrier, which determines the likely rate and size of molecules entering the portal-venous circulation. The intrahepatic effects of this portal-venous inflow of stimulants, as well as platelets on kupffer cells and hepatic stellate cells, drives inflammation, fibrogenesis and carcinogenesis.

Fig. 2: Bile acid (BA) enterohepatic circulation, signalling and related drugs

BAs secreted from hepatocytes (*e.g.* primary BA such as cholic acid [CA]) are undergoing enterohepatic circulation. They are absorbed in the terminal ileum by apical sodium-dependent bile acid transporter (ASBT), leading to fibroblast growth factor (FGF)19-synthesis via farnesoid-X-receptor (FXR)-stimulation. FGF19 on hepatocytes leads to feedback inhibition of *de novo* synthesis of primary BA, via inhibition of the rate limiting enzyme Cyp7A1. The microbiota modulates the BA pool luminally by generating secondary BAs such as deoxycholic acid (DCA), which in the colon passively cross the epithelial barrier. This gives rise to its potential intrahepatic actions, such as secretion of inflammatory and procarcinogenic mediators.

Fig. 3: Physical appearance of Yaq-001 and binding capacity

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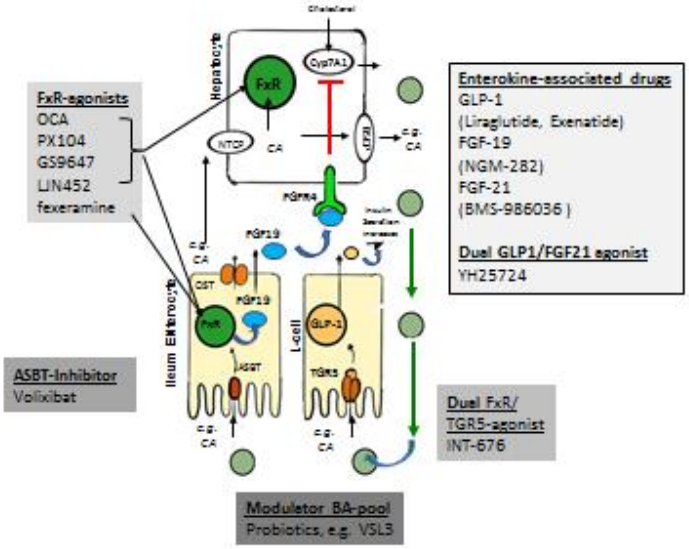
Fig.2


FxR-agonists
OCA
PX104
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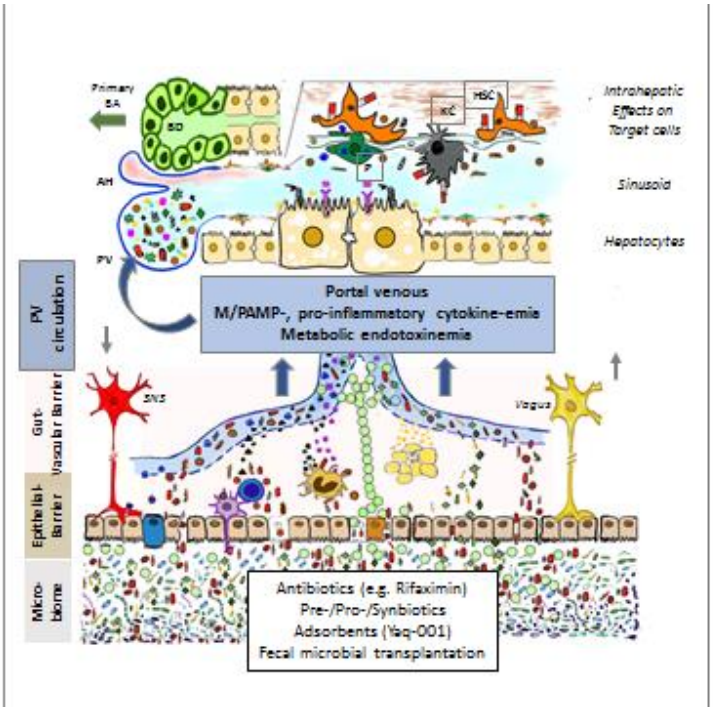
LIN452
fexeramine
Hepatocyte
FxR
Cyp7A1
NTCP
CA
OSTa
e.g. CA
FGFR4
FGF19
Insulin Sensitivity increase
e.g. CA
OST
FGF19
FxR
Ileum Enterocyte
L-cell
GLP-1
TGR5
ASBT
e.g. CA
e.g. CA
Enterokine-associated drugs
GLP-1
(Liraglutide, Exenatide)
FGF-19
(NGM-282)
FGF-21
(BMS-986036)
Dual GLP1/FGF21 agonist
YH25724
ASBT-Inhibitor
Volixibat
Dual FxR/ TGR5-agonist
INT-676
Modulator BA-pool
Probiotics, e.g. VSL3

Fig.1


Primary BA
BD
AH
PV
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Intrahepatic Effects on Target cells
Sinusoid
Hepatocytes
PV circulation
Portal venous
M/PAMP-, pro-inflammatory cytokine-emia
Metabolic endotoxinemia
Gut-Vascular Barrier
Epithelial-Barrier
Micro-biome
SNS
Vagus
Antibiotics (e.g. Rifaximin)
Pre-/Pro-/Synbiotics
Adsorbents (Yaq-001)
Fecal microbial transplantation

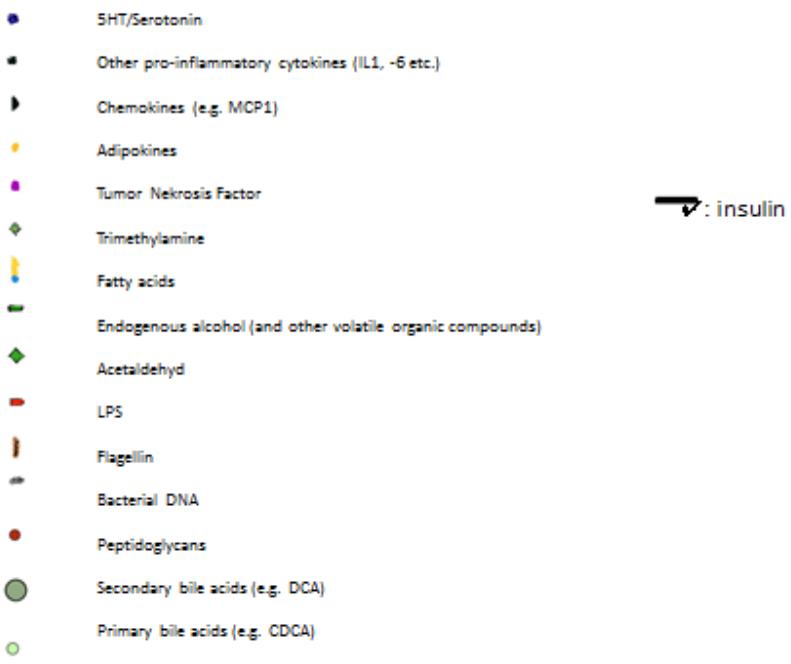
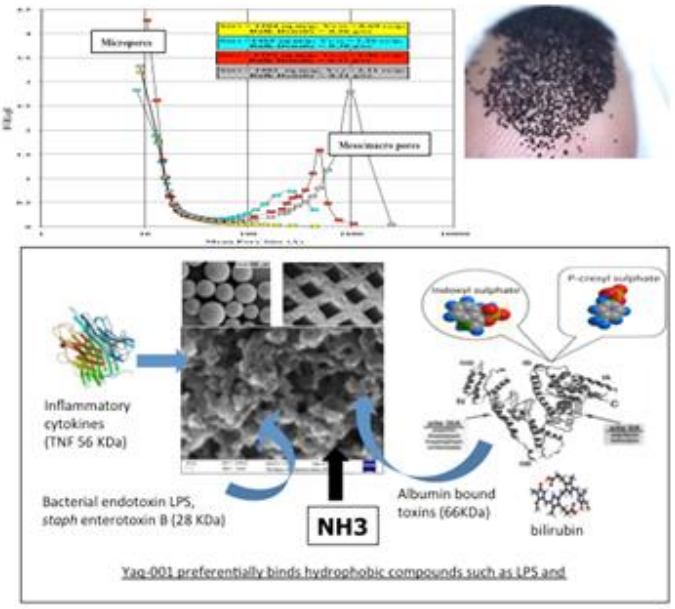


Fig.3

Manufacturing process makes bimodally porous carbons that have both micropores and macropores



...whose macroporosity delivers the power to adsorb large molecules.

Yaq-001 is insoluble. It is excreted with bound molecules attached.