Review

Targeting Gut–Liver Axis for Treatment of Liver Fibrosis and Portal Hypertension

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Abstract: Antifibrotic therapies for the treatment of liver fibrosis represent an unconquered area of drug development. The significant involvement of the gut microbiota as a driving force in a multitude of liver disease, be it pathogenesis or fibrotic progression, suggest that targeting the gut–liver axis, relevant signaling pathways, and/or manipulation of the gut’s commensal microbial composition and its metabolites may offer opportunities for biomarker discovery, novel therapies and personalized medicine development. Here, we review potential links between bacterial translocation and deficits of host-microbiome compartmentalization and liver fibrosis that occur in settings of advanced chronic liver disease. We discuss established and emerging therapeutic strategies, translated from our current knowledge of the gut–liver axis, targeted at restoring intestinal eubiosis, ameliorating hepatic fibrosis and rising portal hypertension that characterize and define the course of decompensated cirrhosis.

Keywords: liver fibrosis; portal hypertension; microbiota; cirrhosis; chronic liver disease; gut–liver axis; bacterial translocation; hepatic macrophages; PRRs; TLRs

1. Introduction

In contrast to acute inflammatory reactions, which are characterized by rapidly resolving vascular changes, edema and neutrophilic inflammation, fibrosis is an intrinsic response to chronic, non-resolving injury and inflammation. The latter triggers a wound healing process that mitigates inflammatory tissue destruction and excessive scarring. Orchestrated by a spectrum of activated extracellular matrix (ECM)-producing cells, protracted injuries often progress towards remodeling and replacement of organ parenchyma by acellular scar tissue accompanied by severe architectural and vascular distortion. Fibrosis is intimately linked to wound healing, serving to maintain organ integrity when tissue disassembly occurs during inflammation, apoptosis, necrosis, and release of lytic enzymes.

The buildup of scar tissue is a hallmark of chronic liver disease (CLD) progression. In the liver, fibrosis is the common endpoint of a plethora of conditions such as chronic viral hepatitis B or C, autoimmune and biliary diseases, alcoholic steatohepatitis (ASH), and a worsening trend of non-alcoholic steatohepatitis (NASH) [1–4]. While mild fibrosis remains largely asymptomatic and usually reversible within days to weeks, its progression towards cirrhosis is a major cause of liver related morbidity and mortality [5–7]. Acute-on-chronic liver disease (ACLD) represents the most advanced stage of liver cirrhosis characterized by acute decompensation of chronic liver disease that can result in multi-system organ failure and a significant short-term mortality [8]. Consequently, cirrhosis and CLD pose a substantial health burden on many countries that has increased at the global scale since the 1990s [5]. In Australia, the age-standardized death rate of cirrhosis in 2016 per 100,000 is 9.6 [9], and the main etiology of decompensated cirrhosis is alcoholic liver disease
With this rapid rise in the burden of liver cirrhosis, there is an immense need to understand the mechanisms of disease pathogenesis and specific targets to reverse or cease fibrosis progression.

Clinically, cirrhosis is associated with progressive liver failure, the risk of hepatocellular carcinoma, and is often accompanied by the development of portal hypertension (PTH). Clinically significant portal hypertension is characterized by hepatic venous pressure gradient (HVPG) $\geq$ 10 mm Hg. PTH triggers many complications, including secondary splanchnic vasodilation and extrahepatic shunt formation resulting in the development of portosystemic collaterals (varices) with significant risk of gastrointestinal bleeding, hyperdynamic syndrome, ascites and hepatic encephalopathy (HE) [10–12]. Severe consequences of PTH can predispose patients to the development of acute decompensation and acute-on-chronic liver failure (ACLF) that is associated with high short-term mortality [13–16].

Currently, there are few therapeutic measures that can prevent progression of clinically significant portal hypertension. Notably, such treatments do not target the main underlying mechanisms and consist of extrahepatic vasoconstrictors (i.e., nonselective beta blockers [NSBBs] [17,18], vasopressin analogues and somatostatin analogues) aimed at ameliorating PTH, or therapies focused on the prevention of PTH-derived complications. Effective artificial liver support remains a major unmet need in patients with end-stage liver disease, with liver transplantation being the only available curative option to date. Organ shortage remains one of the major challenges in liver transplantation and ultimately leads to mortality for those caught on the waiting list.

The bidirectional relationship between the gut and the liver implicates the gastrointestinal microbiome in the development and progression of chronic liver disease [19–22]. While liver-derived bile acids and antimicrobial molecules help shape the gastrointestinal microbiome, the portal vein delivers gut-derived metabolites and microbial products into the liver. Alcohol and diet, two of the main drivers of chronic liver disease, cause significant “local” damage in the liver but are major contributors of microbial dysbiosis in the gut as well as intestinal permeability resulting in microbial translocation into the portal venous system. These factors are exacerbated in end-stage liver disease where bacterial translocation is worsened, combined with impaired hepatic microbial clearance [23]. Conversely, the gut communicates with the liver via close links through the biliary tract, portal vein and systemic circulation.

Growing evidence for the role of gastrointestinal dysfunction in liver disorders is supported by an abundance of evidence from clinical trials demonstrating that liver fibrosis and rising portal hypertension can be efficiently ameliorated by targeting the gut–liver axis [24,25]. Current therapies include pre- and probiotics, and antibiotics to modulate gut microbial composition and intestinal barrier integrity, as well as inhibition of antigen recognition in the liver to limit the local response to microbial products delivered from the gut. This review explores upcoming and state of the art therapeutic strategies for the management of liver fibrosis and portal hypertension translated from our advances in knowledge of the gut–liver axis.

2. The Gut–Liver Axis at the Frontier of Host–Microbial Interactions

The human gastrointestinal tract is the largest barrier surface in contact with the external environment. Therein, the gut microbiota represents a massive microbial ecosystem, harboring upwards of $4 \times 10^{13}$ microbial cells, with a pool of genetic material over one hundred times larger than the human genome, and a metabolic capacity akin to the liver [26–28]. This interdependency that has developed over more than a billion years of mammalian–microbial coevolution has resulted in the entrenchment of our microbiota in every one of our biological systems: the maturation and continued education of the host immune response, selective exclusion of pathogens, regulation of intestinal endocrine functions, neurologic signaling, provision of metabolically available energy sources, vitamins and neurotransmitters, metabolism of bile salts, toxins and drugs and the bidirectional
communication between the gut and other organ systems [29]. This bidirectional crosstalk is best exemplified by the gut–liver axis.

The liver communicates with the intestinal tract through the biliary system and systemic circulation mostly via bile acids (BAs), bioactive mediators and immunoglobulin A antibodies. BAs are amphipathic molecules synthesized from cholesterol in the pericentral hepatocytes. These are conjugated to glycine or taurine and released in the biliary tract. On reaching the small intestine through the duodenum, BAs, together with other biliary components, facilitate emulsification and absorption of dietary fats, cholesterol, and fat-soluble vitamins. About 95% of the BAs are actively reabsorbed in the terminal ileum and transported back to the liver [30,31]. The remaining five percent are deconjugated, dehydrogenated and dehydroxylated by the intestinal microbiota to form secondary bile acids, which reach the liver via passive absorption into the portal circulation. Due to their amphipathic nature, bile acids are toxic for bacterial cells and, thus, exert a strong selective pressure on the microbial populations inhabiting the human gut; they additionally promote the synthesis and secretion of antimicrobial molecules by the intestinal epithelium. This effect helps maintain gut eubiosis and its pool size regulates the microbiome at the highest taxonomic levels.

The term gut–liver axis was coined to highlight the close functional and bidirectional relationship between both these organs resulting from the integration of dietary, metabolic and environmental factors, among others. The present understanding of the many etiologies of liver diseases is underpinned by intestinal dysbiosis and impaired intestinal permeability termed leaky gut [32]. Considerable changes to our diet, alcohol intake, and lifestyle, accompanied with the sanitation revolution and excessive use of antimicrobial agents and medications have drastically accelerated our microbial dysbiosis and augmented systemic inflammation in ways we do not yet comprehend [33].

Intestinal Permeability

The liver receives 70% of its blood supply directly from the gut, where it sits at the crossroad between the portal blood flow coming from the intestinal circulation and peripheral organs. This close anatomical position offers continuous exposure to gastrointestinal antigens, particularly in the context of CLD. These include translocated microbes, microbial products and translocated microbial/pathogen-associated molecular patterns (MAMPs/PAMPs) such as microbial DNA and endotoxins (lipopolysaccharide, flagellin, lipoteichoic acid and peptidoglycan) [34]. Cell death in the gut and liver also generates damage-associated molecular patterns (DAMPs) including adenosine triphosphate (ATP), and intracellular proteins such as heat shock proteins and chromatin associated high-mobility group box 1 (HMGB1) [35].

Liver cell populations including Kupffer cells, hepatic stellate cells (HSCs), sinusoidal cells, biliary epithelial cells, and hepatocytes express innate immune receptors known as pattern recognition receptors (PRRs) that respond to the constant influx of microbial-derived ligands from the gut. When translocated MAMPs/PAMPs reach the liver, they bind PRRs including Toll-like receptors (TLRs) to activate immunomodulatory and inflammatory cascades mediated primarily by signal transducer and activator of transcription (STAT) and nuclear factor-kappa B (NF-κB) transcription factors. These effects are beneficial in the short term by limiting pathogen infection and dispersion, yet detrimental over longer periods of activation by stimulating excessive inflammation, fibrosis and organ damage. The many etiologies of CLD affect gastrointestinal homeostasis by causing changes in the microbiome, innate immune defenses and intestinal permeability. Gastrointestinal dysbiosis, particularly in the context of poor diet or excessive alcohol, inevitably exacerbates chronic liver damage, and is a key target to limit inflammatory and fibrotic progression in CLD patients.

In alcohol-induced liver disease, ethanol impairs intestinal epithelial barrier, elicits intestinal bacterial overgrowth [36] and profoundly disrupts the composition of the microbiome and its metabolome [37]. These effects cumulatively lead to elevated bacterial LPS in portal circulation and can result in rapid systemic endotoxemia. LPS activates Kupffer
cell NF-κB signaling leading to induction of reactive oxygen species (ROS), tumor necrosis factor alpha (TNF-α) and transforming growth factor beta (TGF-β) production. Sustained TNF-α drives mitochondrial dysfunction and neutrophilic infiltration, subsequently triggering inflammation and stimulating apoptosis of hepatocytes. Chronic hepatocyte injury causes release of DAMPS and apoptotic bodies, leading to activation of resident HSCs into myofibroblasts to produce matrix proteins faster than they are degraded. Moreover, the major ethanol metabolite, acetaldehyde, is fibrogenic and causes the release of ROS resulting in paracrine stimulation of HSCs.

In the context of metabolic associated fatty liver disease (MAFLD) [38,39], a two hit [40] theory has been historically adopted to explain the resulting pathogenesis: This theory suggests that hyperglycemia and insulin resistance stimulate the development of hepatic steatosis. The second 'hit' is mediated by lipid-induced cellular stresses such as oxidative stress, apoptosis and gut-derived lipopolysaccharide (LPS) that are required for the development of NASH. More recently, this theory has been considered overly simplistic by ignoring the systemic effects of obesity and has been replaced with the "substrate-overload liver injury model/multi-hit theory [41,42]". Here, surplus fatty acids that develop in MAFLD overwhelm the liver’s metabolic capacity and serve as substrates for the generation of lipotoxic species that provoke endoplasmic reticulum stress and hepatocellular injury leading to a pro-fibrogenic response and genomic instability. In recent years, a line of evidence has suggested a close link between intestinal dysbiosis and the pathogenesis of NAFLD [43–45] (e.g., increased production of intestinal ethanol, bacterial translocation and small intestinal bacterial overgrowth [SIBO]).

Portal pressure is strongly linked to intestinal permeability; venous congestion and splanchnic neoangiogenesis, due to chronic rise of pressure in portal vein, induce phlebectasia, mucosal hypoperfusion and lead to increased permeability in the gut [46]. Abnormal intestinal permeability and bacterial translocation in cirrhotic patients are common and correlated with the degree of portal hypertension.

3. Therapies Targeting the Gut–Liver Axis to Improve Liver Fibrosis and Portal Hypertension

Several interventions targeting the gut–liver axis have been developed in recent years or are otherwise undergoing clinical trials. Here, we will outline current treatments based on their primary target: (1) the intestinal mucosa, (2) the intestinal microbiome, or (3) the hepatic immune response (Figure 1).

3.1. Interventions Targeting the Intestinal Mucosa
3.1.1. FXR Agonists

The regulatory effects of primary BAs have been best studied via their interaction with nuclear receptors such as farnesoid X receptor (FXR) and Takeda G-protein-coupled receptor 5 (TGR5), which modulate hepatic bile acid synthesis, metabolic regulation, inflammation, hepatic fibrosis and vascular homeostasis [47,48]. Obeticholic acid (Ocaliva) (OCA) is a selective semi-synthetic FXR agonist that has been shown to reduce hepatic resistance and portal pressure without systemic effects via increased intrahepatic endothelial nitric oxide synthase activity [49]. Furthermore, OCA has anti-inflammatory properties in vitro, inhibiting pro-inflammatory NF-κB activation in Kupffer cells (KCs) and liver sinusoidal endothelial cells (LSECs) [50]. Moreover, these anti-inflammatory properties reduced HSC activation in rat model of thioacetamide (TAA) fibrosis, as demonstrated by a significant decrease in hepatic alpha-smooth muscle actin (α-SMA) [50]. This data is supported by studies using the synthetic FXR agonist GW4064, which inhibits contraction of HSCs mediated by endothelin-1 [51]. In addition, OCA has been shown to reduce bacterial translocation and attenuate intestinal inflammation in cirrhotic rats by improving the ileal gut-vascular barrier function via antimicrobial peptide induction, improved tight junction expression and reduced loss of fecal albumin [52,53].
Figure 1. The gut–liver axis and its intersection with the intestinal microbiome as a potential therapeutic target for the treatment of liver fibrosis and portal hypertension. The bidirectional relationship between the gut, its microbiome and liver is established via the portal vein which transports immunogenic antigens from the gut. Conversely, the liver feedback route is via bile and antibody secretion in the gut. Our current understanding of the many etiologies of liver diseases is underpinned by intestinal dysbiosis, functional impairment of intestinal barrier, and systemic dissemination of gut MAMPs that trigger an abnormal immune-inflammatory cascade in the liver. Activation of HSCs into proliferative, fibrogenic myofibroblasts is well established as the central driver of hepatic fibrosis. Therapeutic interventions developed or undergoing clinical trials target elements of gut-liver interaction primarily the (1) intestinal mucosa, (2) microbiome and (3) diverse repertoire of immune cell populations in the liver and their sensors.

α-SMA, alpha smooth muscle actin; CTGF/CCN2, connective tissue growth factor; DAMPs, damage associated molecular patterns; HSCs, hepatic stellate cells; IgA, immunoglobulin A; IL-1β, interleukin one beta; IL-6, interleukin six; CCL5, chemokine (C-C motif) ligand 5; HSCs, hepatic stellate cells; KCs, Kupffer cells; LPS, lipopolysaccharides; LTA, lipoteichoic acid; M1, macrophage type1; M2, macrophage type 2; MAMPs, microbe-associated molecular patterns; CCL2/MCP-1, chemokine (C-C motif) ligand 2/monocyte chemoattractant protein-1; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; SCFA, short-chain fatty acids; TMA, trimethylamine; TGFβ, transforming growth factor beta; TNF-α, tumor necrotizing factor alpha.

OCA has recently been examined in a multicenter, double-blind, placebo-controlled, randomized clinical trial (RCT), FLINT (Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment), in patients with non-cirrhotic, non-alcoholic steatohepatitis. When given orally for 72 weeks, OCA improved the histological features of non-alcoholic steatohepatitis and improved liver fibrosis in 45% of patients compared with 23% of patients in the placebo group [54]. Even after one week of treatment, another study by Mookerjee et al. reported...
that nine out of 16 patients with alcoholic cirrhosis receiving OCA responded with a mean HVPG reduction of 28% [55].

Beyond the clinical potential of the first generation of FXR agonists, OCA therapy has been associated with several side effects including high incidences of drug-induced pruritus in both NASH and primary biliary cirrhosis trials [54, 56], increased in low density lipoprotein cholesterol levels and elevated risk of gallstone formation. Most of these side effects are related to its steroidal BA-like chemical structure that enhances some of the TGR5-related side effects. In this context, novel FXR agonists are devoid of TGR5 cross reactivity thus avoiding off-target side effects. EDP-305 is an example of a non-bile acid derivative endowed with FXR agonism/GPBAR1 antagonism that can profoundly inhibit perisinusoidal fibrosis, with over 80% reduction in collagen deposition in methionine choline-deficient (MCD) diet fed mice [57]. EYP001a (Vonafexor, PXL007) is another non-bile acid FXR agonist that is currently being assessed in phase 2 clinical trials for NASH [58] (Enyo Pharma, NCT03812029).

The novel non-steroidal FXR agonist PX20606 has also been shown to improve portal pressure by reducing vascular remodeling while limiting hepatic fibrosis progression, angiogenesis and endothelial dysfunction in rodents [59]. A reduction of bacterial translocation was confirmed by a significant decrease in mesenteric lymph node bacterial count, as well as serum concentrations of lipopolysaccharide binding protein (LBP), TNF and interleukin 6 (IL-6). In cirrhotic animals, PX20606 reduced intestinal fluorescein isothiocyanate (FITC)-dextran uptake (a marker of intestinal permeability) and demonstrated a tendency towards increased ileal zonula occludens 1 (ZO-1) expression, indicating an improvement in gut barrier function due to a reduction in portal hypertensive enteropathy. Another recent study demonstrated that FXR activation stimulates TGR5 expression in the intestinal L cells and drives gut microbiome remodeling to change bile acid composition [60]. This resulted in increased levels of lithocholic acid and tauroliothocholic acid which are potent endogenous agonists for TGR5 (GPBAR1). OCA was found to stabilize the gut–vascular barrier, whereas both FXR agonists abrogated gut–liver translocation of E. coli, highlighting its ability to block microbial transit into the liver [61].

Another novel FXR agonist, Cilofexor (GS-9674 or PX-201), exerts dose-dependent antifibrotic effects and ameliorates portal hypertension in cirrhotic NASH rats [62]. The combination of GS-9674 with the beta-blocker propranolol appeared safe and resulted in an additional decrease of mesenteric hyper-perfusion [62]. Tropifexor (LJN452) is another highly potent FXR agonist, producing robust and dose-dependent reductions in hepatic fat and serum alanine aminotransferase in patients with fibrotic NASH after 12 weeks of therapy based on results from the Novartis, FLIGHT- FXR phase 2b study [63, 64] (Novartis Pharmaceuticals, NCT02855164). In two preclinical distinct rodent models, Tropifexor mediated abrogation of steatohepatitis and fibrosis and induced transcriptome signatures associated with reduction of oxidative stress, fibrogenesis and inflammation [65].

In rats, treatment with the synthetic TGR5 agonist BAR501 for 6 days prior to cannulation of the portal vein reduced the norepinephrine-mediated rise in portal perfusion pressure. Furthermore, administration of the TGR5 agonist inhibited portal hypertension in mice treated for 9 weeks with carbon tetrachloride (CCl₄), while it did not affect fibrosis progression [66]. It was postulated that TGR5 activation promotes the generation and secretion of vasodilatory agents, hydrogen sulfide, and nitric oxide, and inhibits the expression and secretion of the potent vasoconstrictor endothelin-1 from LSECs [67].

3.1.2. Carbon Nanoparticles

Non-absorbable carbon nanoparticles exhibit a high adsorptive capacity for bacterial fragments and represent a novel tool to counteract dysbiosis and translocation of bacterial-derived products. Experimental evidence from a bile-duct ligated cirrhotic rodent model showed that oral therapy with non-absorbable carbon nanoparticles of controlled porosity (Yaq-001) was associated with a significant increase in Firmicutes, particularly Clostridia, and a decrease in Bacteroidetes in stool samples. In addition, this treatment attenuated
LPS-induced ROS production and inflammasome activation by monocytes and neutrophils in bile duct-ligated rats [68].

3.1.3. Duodenal Mucosal Resurfacing

Duodenal mucosal resurfacing (DMR) is a safe, minimally invasive endoscopic procedure that involves a single cycle of circumferential hydrothermal ablation of at least 10 cm of the post-papillary duodenal mucosa [69]. The precise mechanism of action of DMR remains to be determined; however, a recent study by Van Baar et al. demonstrated that DMR can improve liver aminotransferases, decrease hepatocyte mitochondrial and reduce fibrosis-4 scores at 6 months post DMR. These effects were sustained at 12 and 24 months post procedure [70].

3.1.4. Pharmacological Modulation of Gut Peptides

Gut peptides play an important role in relaying signals of nutritional and energy status from the gut. They are released in response to dietary nutrients as well as microbial products and metabolites. Pharmacological modulation of gut peptides holds promise to re-establish metabolic homeostasis in NAFLD and hepatic fibrosis [71,72]. Glucagon-like peptide-1 (GLP-1) is a potent incretin hormone produced and stored by the enteroendocrine L cells of the distal ileum and colon. GLP-1 regulates energy metabolism by promoting glucose-dependent insulin secretion, improving peripheral insulin sensitivity, suppressing glucagon secretion, inhibiting gastric emptying, and promoting satiety. Importantly, several in vitro studies have demonstrated that GLP-1 analogues improve the ability of hepatocytes to handle excess non-esterified fatty acids and lipid production [73].

The LEAN trial (Liraglutide Efficacy and Action in NASH) was the first RCT to document on the efficacy of 48-week treatment period of human glucagon-like peptide-1 receptor (GLP-1R) analogue (Victoza) in adults with biopsy-proven NASH [74,75]. Despite the relatively short duration of the trial, the long-acting GLP-1 receptor agonist Liraglutide histologically reduced active steatohepatitis and lobular inflammation with no worsening of fibrosis from baseline based on Kleiner Fibrosis stage. Recently, Cotadutide (MEDI0382), a dual GLP-1 and glucagon receptor GCGR agonist, has been reported to exert multifactorial reductions in NAFLD activity score, pro-peptide of type III collagen level, fibrosis-4 index to an extent more pronounced than the GLP-1 mono agonist Liraglutide [76] (AstraZeneca, NCT03235050).

The success of the various GLP-1/GCG and GLP-1/GIP (glucose-dependent insulinotropic peptide) dual agonists inspired research towards the development of unimolecular multifunctional peptides with improved plasma half-life and potency that combine agonism for two or more G protein-coupled receptors. The (Glucagon/GIP/GLP-1) GGG tri-agonist (HM15211/LAPS) is a long acting, monomeric peptide triple agonist that is conjugated to the human aglycosylate Fc fragment to extend its circulating half-life. This tri-agonist synergistically reduces liver fat, oxidative stress, and HSC activation (reduced TGF-β and α-SMA gene expression), resulting in greater NAFLD activity score (NAS) reduction than GLP-1RA, apoptosis signal-regulating kinase 1 inhibitor, or a FXR agonist in an MCD mouse model [77]. In line with these results, the GGG tri-agonist HM15211 is currently being evaluated for NASH and fibrosis as part of a Phase 1b/2a clinical trial. Preliminary data have demonstrated improvement of hepatic steatosis, inflammation and fibrosis [78]. Phase 1 clinical trials using ALT-801 (Altimmune, Inc., NCT04561245) and DD01 (Neuraly, Inc. NCT04812262) as well as Phase 2 clinical trials of Efinopegduutide (IN) 64565111/HM12525A) (Merck Sharp & Dohme Corp., NCT04944992) and BI456906 (Boehringer Ingelheim, NCT04771273), all dual GLP-1/Glucagon receptor agonists, are underway in patients with histologically proven NASH, underlining the utility of this approach.

Other GLP-1 analogs were similarly effective in the treatment of NASH but failed to resolve fibrosis. Phase 2 trials using the long-lasting GLP-1 analog Semaglutide (Ozempic) for 72-week in patients with biopsy-confirmed NASH and liver fibrosis of stage F1, F2, or
F3, resulted in NASH resolution but unexpected lack of improvement of fibrosis stage [79] (Novo Nordisk A/S, NCT02970942). Similarly, results from (D-LIFT trial [80]) demonstrated that Dulaglutide significantly reduced liver fat content and improved GGT levels in participants with NAFLD but was unable to reduce liver stiffness and transaminases.

Fibroblast growth factor 19 (FGF19) is a hormone produced by enterocytes of the terminal ileum in response to bile acid-mediated FXR activation to regulate bile acid synthesis. Therefore, FXR agonists such as OCA possess potent dual activity by acting directly on the liver and indirectly via FGF19 [81]. From a clinical standpoint, it is challenging to identify whether the multi-faceted and anti-fibrotic effects of FXR agonists are due to FXR activation in the liver or the effects of FGF19 from the gut. In a proof-of-concept study, an engineered FGF19 analog (Aldafermin) was administered for up to 24 weeks in 53 patients with histologically confirmed NASH. It reduced liver fat and improved liver fibrosis (≥1-stage decrease in fibrosis score) in 38% of patients versus 18% in the placebo group [82] (NGM Biopharmaceuticals, Inc., NCT02443116).

Another member of the FGF subfamily is fibroblast growth factor 21 (FGF21). FGF21 is a pleiotropic hormone produced mainly by hepatocytes in a PPAR-alpha regulated fashion, acting as regulator of energy balance, glucose and lipid homeostasis via a heterodimeric receptor consisting of FGF receptor 1 (FGFR1) and β-klotho [83]. FGF21 represents an intriguing target for modulation of NASH and progression to advanced fibrosis. Data obtained from the phase 2 clinical trial using the PEGylated human FGF21 analogue, Pegbelfermin (BMS-986036) administered for 16 weeks for patients with non-alcoholic steatohepatitis (fibrosis stage 1–3) resulted in significant reduction in hepatic fat as measured by Magnetic Resonance Imaging Proton Density Fat Fraction—MRI-PDFF, PRO-C3 (a fibrosis biomarker), and improvement of metabolic parameters (adiponectin and lipid concentrations) [84] (Bristol-Myers Squibb, NCT02413372). In view of this, a handful of drugs using the enterokine pathway are in the clinical pipeline, including Efruxifermin (AKR-001), another FGF21 mimetic. In Phase 2 studies in non-alcoholic steatohepatitis (NASH) patients with F1-F3 fibrosis, Efruxifermin demonstrated effective reduction of liver fat content with 48% of treatment responders achieving an improvement in fibrosis stage [85] (Akero Therapeutics, Inc. NCT03976401, NCT04767529).

In summary, there are a variety of therapeutic interventions targeting the intestinal mucosa for the treatment of advanced liver disease. Figure 2 summarizes several of these interventions that aim to restore homeostasis within the gut–liver axis.

3.2. Interventions Targeting the Intestinal Microbiome

Individuals with liver fibrosis have a markedly altered microbial diversity, characterized by a decline in microbial gene richness and function [86,87]. Perturbations in bacterial metagenomic and metabolomic signatures and their association with liver disease suggests that manipulation of commensal microbial composition or function is essential to restore homeostasis [88–90]. Therapies that aim to achieve restoration of the intestinal microbiome include selected combinations of metabolites (postbiotics) produced by the microbiome that are generated from dietary components, as well as probiotics and prebiotics that are used to stimulate the growth of “good bacteria”. Alternatively, antibiotics and fecal microbiota transplantation are used to broadly remove/replace the majority of the microbial ecosystem and are often used in combination with gentler approaches (pre-/probiotics) to recolonize the gut (Figure 3).
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**Figure 2.** Interventions targeting intestinal mucosa. FXR agonists—In Kupffer cells (KCs) and liver sinusoidal endothelial cells (LSECs), FXR induction leads to the expression of short heterodimeric partner (SHP), and further downregulation of cholesterol 7a-hydroxylase CYP71A. FXR agonists: Cilofexor, CW4064, EDP-305, EYP001a, OCA (Obeticholic acid), PX20606, Tropifexor. Carbon nanoparticles—Non-absorbable carbon particles exhibit a high adsorptive capacity for bacterial-derived products, counteracting bacterial translocation. Duodenal mucosal resurfacing (DMR) is a minimally invasive upper endoscopic procedure that involves circumferential mucosal lifting followed by hydrothermal ablation of duodenal mucosa. Pharmacological modulation of gut peptide agonists of mucosal gut receptor, including GLP-1 agonists, GLP-1/GCG agonists and tri-agonists (GLP1/GCG/GIP), are all intriguing drugs for modulation of fibrosis. Aldafermin (an engineered FGF19 analog), Pegbelfermin (PEGylated human FGF21 analogue) and Efruxifermin (FGF21 mimetic) represent promising targets for modulation of liver fibrosis. GLP-1R, glucagon-like peptide-1 receptor; GCGR, glucagon receptor; GIPR, glucagon-like peptide-1 receptor.
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Figure 3. Interventions targeting the intestinal microbiome. Advances in our knowledge of the gut–liver axis are driving the development of therapeutic tools based on microbiota composition and its metabolites (postbiotics). Modifying intestinal content with non-absorbable antibiotics (Rifixamin, Norfloxacin) or specific pro- pre- or synbiotics (VSL#1, lactulose, SYNB1020) or target fecal microbiota transplantation are increasingly recognized in clinical trials as effective interventions targeting the microbiome to effectively treat liver disease. SCFAs, short-chain fatty acids; TAM, trimethylamine; Uro-A, Urilothlin-A.

3.2.1. Targeting Microbiome Composition

(a) Non-absorbable Antibiotics. Patients with cirrhosis are predisposed to intestinal dysmotility, bacterial overgrowth, and increased intestinal permeability, all leading to an increase in bacterial translocation (BT) and increased endotoxemia. In cirrhosis, there is an increased relative abundance of bacterial taxa belonging to Enterobacteriaceae (Gram-negative (-) rods such as Escherichia coli (E. coli) and Klebsiella), Enterococcaceae (Enterococcus faecalis and E. faecium), and Streptococcaceae, combined with a lower abundance of potentially beneficial autochthonous taxa such as Lachnospiraceae Ruminococcaceae, and Clostridiales XIV in advanced cirrhosis [86]. The invasion of oral bacteria (such as Prevotella or Veillonella) into the distal intestine is also observed in cirrhotic patients [91,92].

In addition to changes in microbiome composition, PTH damages the intestinal barrier and thus increases microbial translocation into the portal system. A surrogate marker of BT, LBP, was observed to be increased in 42% of cirrhotic patients [93]. In addition, up to 30.8% of patients with Child-Pugh C cirrhosis have positive bacterial cultures of mesenteric lymph nodes compared to 8.6% of non-cirrhotics [94]. BT has also been associated with other portal hypertension related complications such as HE and spontaneous bacterial peritonitis (SBP) [95]. In recent years, an association between bacterial infection and portal hypertension in cirrhosis has been established. Bacterial infection is an independent predictor of the occurrence of variceal hemorrhage (VH) and is also the strongest independent factor associated with failure to control VH, earlier re-bleeding, coagulation abnormalities...
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and mortality [96]. Hence, current guidelines recommend continuous prophylaxis with antibiotics to protect against the development of decompensation events such as SBP, either as primary prophylaxis in specific conditions or as secondary prophylaxis after an episode of SBP. Meanwhile, third-generation cephalosporins and fluoroquinolones, are recommended for prophylaxis of variceal bleeding. A study by Moghadamrad et al. showed that wild type mice colonized with intestinal microbiota presented with significantly higher portal pressure levels after partial portal vein ligation, when compared to germ free mice [97]. Consequently, the effect of antibiotic therapy on portal pressure has become heavily investigated in human trials.

The administration of antibiotics can eliminate dysbiosis and pathobionts, and additionally reduces enteric production of inflammatory cytokines, stabilizes the gut barrier and decreases the production of harmful secondary bile acids [98]. Rifaximin is a non-absorbable entero-selective broad-spectrum antibiotic that remains relevant in this context, even since its approval over 30 years ago in 1987 [99]. Rifaximin is beneficial for HE in cirrhosis and is currently recommended by the European Association for the Study of Liver [100] and the American Association for the Study of Liver Diseases (AASLD) [101] as one of the first-line drug for PTH therapy and prophylaxis. Kaji et al. demonstrated that Rifaximin ameliorates HE and lowers endotoxemia with minimal change in microbiome composition [102]. Moreover, Rifaximin is recommended as add-on therapy to lactulose for prevention of overt HE according to AASLD and EASL guidelines.

Unfortunately, the hydrophobic nature of Rifaximin makes it largely insoluble in water, and it requires BA for adequate solubilization. A newer formulation termed Rifaximin soluble solid dispersion (SSD) is water soluble and is of therapeutic benefit for patients with advanced liver disease who have lower intestinal BA concentration. A phase 2 study concluded that oral Rifaximin SSD treatment in patients with early decompensated cirrhosis could reduce all-cause hospitalization or mortality [103] (Bausch Health Americas, Inc. NCT01904409).

Rifaximin is believed to reduce hepatic fibrosis progression by improving intestinal permeability by increasing intestinal ZO-1 expression. In a murine model of bile duct ligation-induced liver fibrosis, Zhu et al. demonstrated that Rifaximin reduced fibrosis, angiogenesis and portal hypertension via inhibition of TLR4 pathway activation [104]. As expected, both aerobic and anaerobic fecal bacteria counts, which were increased after bile duct ligation, were significantly reduced in animals receiving Rifaximin.

A small cohort study by Vlachogiannakos et al. in 2009 demonstrated that HVPG values decreased significantly after intestinal decontamination with Rifaximin for 28 days in patients with alcohol-related decompensated cirrhosis [105]. Furthermore, long-term use of Rifaximin reduced the risk of developing complications of PTH and improved survival. On the contrary, a recent randomized, double blinded, placebo-controlled trial investigating the hemodynamic effect of Rifaximin in 54 patients with cirrhotic ascites without signs of overt HE observed no difference in HVPG compared to placebo [106]. A possible explanation for this discrepancy is due to Rifaximin’s effect on the gut microbiome. It was hypothesized that Rifaximin limits HE development by stimulating the growth of colonic microbes that produce less ROS and amino acids (Copenhagen University Hospital, Hvidovre, NCT01769040).

Rifaximin has also proven useful when combined with non-selective beta-blockers. NSSBs function to prevent rebleeding by decreasing cardiac output, as well as inducing splanchnic arterial vasoconstriction and therefore reducing splanchnic blood flow. They have additionally been shown to improve intestinal permeability in cirrhosis and consequently decreased bacterial translocation. A large clinical trial investigating the hemodynamic response of Rifaximin and propanol combination therapy versus propanol monotherapy on complications of decompensated cirrhosis and portal hypertension showed that Rifaximin combination therapy with propanol has an additive effect in improving PTH [107].

A recent study in a rat model of NASH by Fujinaga et al. showed that the combination of
an angiotensin-II receptor blocker (ARB) and Rifaximin showed a stronger inhibitory effect compared to that conferred by a single agent [108].

Norfloxacin is a synthetic broad-spectrum antibiotic, and poorly absorbed fluoroquinolone, that has been used to achieve selective intestinal decontamination in cirrhotic patients. Treatment with Norfloxacin has been shown to reverse the hyperdynamic state, albeit without an effect on HVPG [109,110]. Norfloxacin nonetheless seems to improve survival in cirrhotic patients with reduced ascitic fluid protein concentrations and decrease risk of AD and ACLF [111]. In a small RCT, selective intestinal decontamination with Norfloxacin partially reversed the hyperdynamic circulatory state in cirrhotic patients with a reduction of serum LPS [112]. Furthermore, a RCT has shown that Norfloxacin, when combined with standard medical therapy improved survival in patients with decompensated alcoholic cirrhosis and liver failure (Assistance Publique—Hôpitaux de Paris, NCT01037959).

(b) Probiotics, prebiotics and synbiotics. Antibiotic regimens cause a lasting disruption to the composition of the gut microbiome, opening the doors to antibiotic resistance. Consequently, the use of pre-, pro- and/or synbiotics has long been advocated for restoration of intestinal microbial diversity. Prebiotics are substrates that are selectively used by host microorganisms conferring a health benefit [113] (International Scientific Association for Probiotics and Prebiotics-ISAPP consensus 2016), while probiotics are “live microorganisms that, when administered in adequate amounts confer a health benefit on the host [114] (Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO)-ISAPP 2013). Synbiotics are a synergistic combination of probiotics and prebiotics, which serve to improve the therapeutic benefits of probiotics by combining them with prebiotics to enhance their growth in the colon. The therapeutic and prophylactic effects of probiotics can be predetermined by modifying bacteria to produce biotherapeutic metabolites, and immune-modulating compounds to enhance host immunity and barrier integrity in the form of post-biotics. The beneficial effects induced by pre-, pro-, synbiotics are largely individual, and dependent on host genetic background, diet and gut microbial milieu.

In randomized control trial VSL#3, a live formulation of eight bacterial species (four strains of Lactobacillus, three strains of Bifidobacterium (Bifidobacterium breve, longum, and infantis), and one strain of Streptococcus) reduced the risk of hospitalization for HE in patients with cirrhosis [115]. VSL#3 treatment stimulated an increase in plasma albumin and hemoglobin, which can lead to lower MELD scores in patients with decompensated liver cirrhosis. In addition, a long-term investigation of 39 patients with biopsy-proven NAFLD demonstrated that VSL#3 (12 strains, 675 billion colony forming units (CFU)/day) administered for one year significantly improved NAS, and resulted in significant improvement in hepatocyte ballooning and hepatic fibrosis [116].

Upon examining all available clinical evidence, the impact of VSL#3 on HVPG remains uncertain and has led to reservations on use of probiotics in management of portal hypertension. One study of 12 patients demonstrated that administration of the probiotic mixture VSL#3 improved the hepatic and systemic hemodynamics and serum sodium levels in patients with cirrhosis [117], while two additional studies of a similar size showed that VSL#3 did not impact HVPG in both compensated and decompensated patients with cirrhosis [118,119]. When combined with the beta blocker propranolol, the VSL#3 probiotic mixture was safe and well tolerated in patients with cirrhosis and improved the response rate of propranolol with respect to HVPG [120].

Apart from their production of beneficial metabolites, probiotics can also be used to consume harmful bacterial products. SYNB1020, an engineered Escherichia Coli Nissle strain has been designed to consume colonic ammonia in patients with cirrhosis [121] (Synlogic, NCT03447730). Capturing part of gut the ammonia can attenuate clinical symptoms of hyperammonemia in conditions like urea cycle disorders and HE. Its development has unfortunately been discontinued given the negative trial data from an interim analysis of a placebo-controlled phase 1b/2a.
The benefits of prebiotics have been known since many years ago. Prebiotics, such as inulin, were associated with an increase in short-chain fatty acids such as propionate in the colon and portal vein. Lactulose, a non-absorbable disaccharide effectively reduces ammonia absorption in the gut and is an effective treatment for HE \[101,122,123\]. Despite its widespread use as a laxative and prebiotic, its influence on gut microbiota composition remains undefined. Lactulose acidifies the colonic contents resulting in decreased passive non-ionic diffusion of ammonia into the systemic concentration, as well as reduced formation of toxic SCFAs. Furthermore, lactulose prompts the growth of non-urease-producing bacteria such as lactobacillus and bifidobacteria.

(c) Fecal microbiota transplantation. Fecal microbiota transplantation (FMT) is used to replenish a healthy gut microbial environment and restore physiological colonization by transfer of microbial flora from a healthy donor. It represents a more robust method of manipulating the gut microbiota as compared to dietary/probiotic treatments and is now an accepted therapy for recurrent or refractory Clostridium difficile infection. A phase 1 study showed that FMT with oral capsules, following antibiotic pre-treatment with Rifaximin, was well tolerated and safe long term, and was associated with a reduction in serum LBP, and improved mucosal barrier integrity and EncephalApp performance in patients with cirrhosis and recurrent HE with MELD < 17 \[124\]. This is consistent with a RCT of 21 NAFLD patients provided with allogenic FMT, demonstrating improved intestinal barrier function, albeit no change in steatosis or insulin resistance \[125\]. Additional beneficial effects of FMT on the liver have been demonstrated in rats. In a model of non-alcoholic steatohepatitis with portal hypertension, transplantation of stool from healthy animals significantly reduced HVPG by 31% and restored the sensitivity to insulin via the hepatic protein kinase B-dependent endothelial nitric oxide synthase signaling pathway \[126\].

Surprisingly, FMT has also demonstrated promise as a measure for limiting alcoholic cirrhosis progression. In a phase 1 RCT of 20 patients, FMT enema from a donor enriched in Ruminococcaceae and short-chain fatty acid-producing taxa Lachnospiraceae was associated with short-term reduction in alcohol craving and consumption in patients with alcohol-associated cirrhosis (Hunter Holmes Mcguire Veteran Affairs Medical Center, NCT03416751). These data hint at a particularly potent effect of FMT in restoring microbiota composition and functionality in the course of alcoholic liver disease. FMT success is likely to be dependent on functionality of particular microbial consortia. Indeed, FMT has increased relative abundance of butyrate-producing genera such as Roseburia and Odoribacter, which are typically reduced during alcoholic cirrhosis \[127\]. It is thought that SCFA modulation along with an increase in beneficial taxa engages the gut–brain axis and hence could explain the reduction in alcohol craving.

Despite the clinically evident success and safety of FMT, it remains a second-line treatment owing to the risk of disease transmission between the donor and recipient, undesirable side effects, sustainability of the post-FMT microbiota, and the unclear effects on the recipient’s immune system. Further rigorous clinical studies are warranted to determine the utility of FMT in liver fibrosis. Table 1 summarizes ongoing pro-, pre-, synbiotic and FMT clinical tails for treatment of liver disease.

3.2.2. Postbiotics

The gut is the residence for up to 80% of immune cells in the body \[128\], where they respond to bacterial metabolites (postbiotics) responsible for immune system ontogeny, modulation of immune signaling and intestinal mucosal integrity. Postbiotics potentiate the morphological structures of the intestinal barrier by increasing the expression of tight junction proteins ZO-1 and intestinal mucin levels and increasing the secretion of anti-inflammatory cytokines such as IL-10 \[129\]. This protective role extends to the liver, as demonstrated by increased susceptibility to liver fibrosis in germ-free mice \[130\].
### Table 1. Summary of ongoing pre-, pro, synbiotics and FMT clinical trials for treatment of liver disease.

<table>
<thead>
<tr>
<th>ClinicalTrials.gov Identifier and Sponsor</th>
<th>Study</th>
<th>Estimated Completion</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02642172, Kaplan Medical Center, Israel</td>
<td>Evaluating whether prebiotics—ITF (Inulin/OFS 75/25) are effective in treating patients with NFALD.</td>
<td>2023</td>
</tr>
<tr>
<td>NCT0256860, University of Calgary, Canada</td>
<td>Effect of prebiotic fiber oligofructose-enriched inulin (Synergy1) supplementation, in conjunction with diet-induced weight loss, on reduction of liver fat and injury.</td>
<td>2022</td>
</tr>
<tr>
<td>NCT03467282, Hospital de Clinicas de Porto Alegre, Brazil</td>
<td>Probiotic supplementation (Lactobacillus acidophilus, Bifidobacterium lactis, Lactobacillus rhamnosus and Lactobacillus paracasei) in nonalcoholic steatohepatitis patients (PROBILIVER trail).</td>
<td>2021</td>
</tr>
<tr>
<td>NCT03863730, Odense University Hospital, Denmark</td>
<td>Prevention of progression in alcoholic liver disease by modulating dysbiotic microbiota by Profermin Plus, FSMP (food for special medical purposes), probiotics (based on fermented oats, Lactobacillus plantarum 299v, barley malt and Lecithin plus Thiamin) (SYN-ALD).</td>
<td>2021</td>
</tr>
<tr>
<td>NCT04175392, William Beaumont Hospitals, USA</td>
<td>Effect of probiotics (Align) in non-alcoholic fatty liver disease and steatohepatitis measured by transient elastography (PRONE Study).</td>
<td>2023</td>
</tr>
<tr>
<td>NCT04671186, Northwell Health, USA</td>
<td>Role of probiotics (Culturelle (Lactobacillus rhamnosus strain GG)) in treatment of pediatric nonalcoholic fatty liver disease (NAFLD) patients by assessing with fibroscan.</td>
<td>2021</td>
</tr>
<tr>
<td>NCT03749070, Camila Ribeiro de Avelar, Brazil</td>
<td>Effect of Silymarin (dietary supplement) on clinical evolution and nutritional variables of patients with non-alcoholic fatty liver disease.</td>
<td>2021</td>
</tr>
<tr>
<td>NCT04871360, Universidad de Guanajuato, Mexico</td>
<td>Effect of oral L-Citrulline supplementation on liver function and non-alcoholic fatty liver disease in adolescents with obesity.</td>
<td>2021</td>
</tr>
<tr>
<td>NCT04988805, Naga P. Chalasani, Indiana University School of Medicine, Indiana USA</td>
<td>The effect of Vitamin E and Docosahexaenoic Acid Ethyl Ester on non-alcoholic fatty liver disease (NAFLD).</td>
<td>2022</td>
</tr>
<tr>
<td>NCT04823676, Hospital General Dr. Manuel Gea Gonzalez Mexico city, Mexico</td>
<td>Efficacy and safety of a probiotic composition (mixture of two Lactoplantibacillus plantarum strains (formerly Lactobacillus plantarum) and one Levilactobacillus brevis strain (formerly Lactobacillus brevis), in a maltodextrin carrier (E1400)) as adjunct treatment in the comprehensive management of metabolism-associated hepatic steatosis in adults.</td>
<td>2022</td>
</tr>
<tr>
<td>NCT04781933, Mativa-Tech SA, France</td>
<td>“Combo” (a combination of dietary supplements including probiotics (Lactobacillus rhamnosus GG, Bifidobacterium breve BR03, Lactobacillus plantarum) and Glutamine, Quercetin, Vitamin E, Curcumin, Silybin, Pectin) in NASH improvement (ICAN).</td>
<td>2022</td>
</tr>
<tr>
<td>NCT03897218, 1. Medical University of Vienna, Austria 2. University Hospital RWTH Aachen, Germany 3. Sahlgrenska University Hospital, Sweden</td>
<td>Dietary modulation of intestinal microbiota as trigger of liver health: Role of bile acids—“A Diet for Liver Health” (ADLH) using oatmeal flakes with prebiotic food supplements.</td>
<td>2022</td>
</tr>
<tr>
<td>NCT04465032, Leiden University Medical Center, The Netherlands</td>
<td>The effect of consecutive fecal microbiota transplantation on non-alcoholic fatty liver disease (NAFLD). Fecal transplantation will be performed via gastroduodenal endoscopy of autologous vs allogenic (lean donor) at 3 and 6 weeks (NAFTx).</td>
<td>2021</td>
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</table>

Gut commensal microbes produce a myriad of metabolites that modulate their environment. These include short-chain fatty acids (SCFAs) such as acetate, propionate and butyrate, which are end products of bacterial fermentation of dietary fibers, proteins with immunomodulatory activities (e.g., bacterial p40, HM0539), biosurfactants, bacteri-
ocins, polysaccharides, and vitamins, to name a few. Given the versatility of postbiotics, meticulous clinical trials are required to support their use in diseases of gut-barrier dysfunction. Nonetheless, there remains a significant amount of evidence demonstrating the efficacy of bacterial metabolites as a treatment, as well as their dietary precursors and metabolizing microbes.

(a) Choline. Choline is an essential macronutrient with many functions, ranging from lipid metabolism and neurotransmitter synthesis to cell structure and DNA methylation [131]. The gut microbiota metabolizes dietary choline into trimethylamine (TMA), which enters into the portal circulation where it is oxidized by hepatic flavin-containing monoxygenases in the liver, forming trimethylamine-N-oxide (TMAO) [132]. This conversion of choline into methylamines results in deficiency of phosphatidylcholine, one of the major cytoprotective components of hepatocyte membranes against bile salts. Furthermore, the microbial TMA metabolite TMAO has been strongly associated with the presence and severity of NAFLD [133]. TMAO is thought to aggravate liver steatosis via modulation of the bile acid pool and suppression BA-mediated hepatic FXR signaling. Metagenomic analysis of stool microbiome of pediatric NAFLD patients revealed increased abundance of Gammaproteobacteria and Prevotella in comparison with the microbiota of obese children without NAFLD [134]. The class Gammaproteobacteria is known to harbor high concentrations of choline-metabolizing enzymes, which can influence susceptibility to and progression of hepatic steatosis.

(b) Short-chain fatty acids. Short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate are anaerobic fermentation products generated by cecal and colonic microbiota from non-digestible carbohydrates such as non-starch polysaccharides, resistant starch, and miscellaneous low-digestible saccharide prebiotics. Research has predominantly been focused on the least abundant SCFA, which perhaps possesses the most important biological roles—butyrate. Butyrate is a primary enterocyte energy source that dynamically promotes the maintenance of the colonic barrier via induction of tight junction proteins and mucins [135,136]. Moreover, butyrate exerts an anti-inflammatory effect and can suppress colonic and hepatic LPS-induced production of pro-inflammatory cytokines via inhibition of NF-κB activation. Studies suggest that butyrate produced by intestinal microbiota can modulate the pathogenesis of liver fibrosis. Butyric acid has been shown to be inversely correlated with the model for end-stage liver disease (MELD) score and was further reduced in patients with history of ascites, HE, and SBP [137]. Of note, the fraction of SCFA-producing bacterial phyla such as *Firmicutes* and *Bacteroidetes* are diminished during advanced stages of liver cirrhosis. Moreover, chronic alcohol intake induces skewing of intestinal SCFA concentrations, increasing the luminal acetate: butyrate ratio. Butyrate when supplemented in the form of rapidly absorbing prodrug, Tributyrin, to mice on chronic binge alcohol exposure, altered alcohol-induced intestinal permeability, inflammatory cytokine expression and liver transaminases when administered to mice following chronic alcohol exposure [138]. Icosabutate is a structurally engineered fatty acid that selectively targets the liver through the portal vein. Preliminary data presented at the International Liver Congress 2021 from an ongoing phase 2 study of patients with biopsy-confirmed NASH are encouraging. A 4-month treatment with Icosabutate caused significant dose-dependent decreases in alanine transaminase, aspartate transaminase, gamma-glutamyltransferase, and alkaline phosphatase combined with significant reductions in fibrosis PRO-C3 and Enhanced Liver Fibrosis (ELF) scores [139] (NorthSea Therapeutics B.V., NCT04052516).

(c) Urolithin A. Among the metabolites of hydrolysable tannins that are produced in the gut microbiome, urolithin A (UroA) has received enormous attention recently as a novel candidate with anti-inflammatory and antioxidant effects in vitro and in vivo. UroA is believed to enhance gut barrier function by inducing tight junction proteins (Occludin, Claudin-4 and ZO-1) via activation of the aryl hydrocarbon receptor [140]. UroA demonstrates potent anti-inflammatory activity, reducing LPS-mediated IL-6 and TNF production via NF-κB suppression. In a recent study, UroA has been shown to attenuate ALD pathogenesis in both acute and chronic experimental mouse models by
reducing alcohol induced barrier permeability, systemic endotoxin levels and inflammatory mediators [141].

4. Interventions Targeting Hepatic Immune Response

The progression of chronic liver disease is mediated primarily by the hepatic insult that triggers chronic immune activation, inflammation and fibrosis. The etiologies responsible for CLD and its related pathologies (e.g., portal hypertension and biliary dysfunction) also stimulate gastrointestinal dysfunction and significant alterations in the microbiome, allowing antigens to transit into the liver where they can exacerbate inflammatory conditions. Consequently, numerous therapeutic strategies are currently under development targeting the diverse repertoire of immune cell populations in the liver and their sensors responsible for antigen recognition and immune cell activation. The abundance of cell-surface, cytoplasmic and nuclear molecules that contribute to HSC activation provide fertile ground for novel antifibrotic therapies, several of which are undergoing drug development and clinical trials. A detailed cataloguing of these approaches is beyond the scope of this review and can be reviewed elsewhere [142]. This section will outline the mechanisms by which gastrointestinal and hepatic antigens exacerbate the progression of CLD, and current treatments aimed to quench the hepatic immune response. We pay special attention to liver macrophage populations due to their central role in the initiation and exacerbation of chronic liver disease and HSC activation.

4.1. Targeting Pattern Recognition Receptors

PRRs are a diverse group of sensors capable of recognizing molecular patterns conserved among microbial species, termed PAMPs [143]. In addition, they recognize an ever-growing list of endogenous molecules released following cellular damage/death called DAMPs. The first-discovered and best-characterized PRR families are TLRs. TLRs are evolutionarily conserved type I transmembrane proteins, expressed in many internal organs including the liver. At present, 13 human TLRs have been identified that recognize diverse intracellular and extracellular microbial antigens ranging from DNA and RNA to bacterial membrane and fungal wall components. In addition to TLRs, a variety of PRRs have been characterized, including cell surface c-type lectin receptors (CLRs), as well as intracellular receptors such as the family of nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene I (RIG-I), stimulator of interferon genes (STING).

As outlined above, a compromised gut barrier in CLD allows influx of gut-derived antigenic loads via the portal vein, triggering chronic breakdown in TLR tolerance against endogenous ligands and further transcriptional expression of pro-inflammatory/anti-inflammatory mediators and interferons. This inflammatory milieu/micro-environment in the liver results in activation of quiescent HSCs to initiate the production of several extracellular matrix proteins including collagen. In liver injury and hepatic fibrogenesis TLR3, TLR4 and TLR9 have been best characterized with respect to inflammation and fibrosis resulting from gut-derived PAMPs and host-derived DAMPs. Innate immune sensing of gut-derived microbial products by PRRs and their impact on chronic liver disease have been recently reviewed elsewhere [144–146]. Herein, we will focus on the PRRs for which antifibrotic treatments are in development, including TLR3, 4, and 9, as well as the NLRP3 inflammasome.

4.1.1. Key Toll-like Receptors in Liver Fibrosis

(a) Toll-like Receptor 4. TLR4 in combination with its co-receptors MD2 and CD14 recognize potent inflammatory PAMPs (flagellin and LPS) and endogenous DAMPs such as calprotectin, S100A8/9 HMGB1 and HSP70 [147]. In the liver, TLR4 is ubiquitously expressed by both hepatocytes and non-parenchymal cells, including LSECs and KCs. Importantly, the activation of TLR4 is directly linked to circulating LPS, hepatic inflammation and fibrosis development. Activated Kupffer cells secrete pro-inflammatory cytokines

...
(TNF, IL-1β, IL-6) and fibrogenic stimuli (TGF-β, platelet-derived growth factor [PDGF]) to stimulate HSC differentiation into extracellular matrix producing myofibroblasts [148,149]. TLR4 stimulation additionally leads to upregulation of inflammatory cytokines (TNF, IL-1β, IL-6) and chemokines (such as MCP-1, MIP-1β, and RANTES) in HSCs, further recruiting monocytes and KCs [150]. During chronic LPS stimulation this positive feedback loop is potentiated by NFκB mediated repression of Bambi transcriptional activity which contributes to TLR4-mediated enhancement of TGF-β signaling in HSCs [149,151].

Recent studies in murine models have demonstrated that TLR4 deficiency reduces pro-inflammatory cytokine production of IL-1α, IL-1β and IL-6 as well as liver injury in acetaminophen-induced liver injury and ALD [152]. In addition, endotoxin-resistant TLR4 mutant mice fed the (MCD) NASH diet possessed significantly reduced hepatic inflammation and injury markers supporting the pro-inflammatory role of TLR4 [153]. Consequently, the prevention of excessive activation and inhibition of TLR4 have become attractive pharmacological strategies to inhibit fibrogenesis.

Although many TLR4 antagonists have been examined, very few have progressed into clinical trials due to worries regarding potential effects on systemic immunity. Nonetheless, both animal models and in vitro studies have demonstrated a clear benefit of TLR4 antagonism and in multiple etiologies of CLD. The most well-known TLR4 antagonist to enter clinical trials was Eritoran, followed by TAK-242 and NI-0101. Eritoran tetrasodium (E5564) is a synthetic Lipid A mimic that binds to the MD2-TLR4 interface. It has been reported to decrease LPS-induced acute severe liver injury in rats by decreasing activation of MAP kinases and TNF gene expression [154,155]. Inhibition of TLR4 with the antagonist, E5564 was tested in humans with severe sepsis in the ACCESS trial, showing no effect on 28-day all cause mortality [156]. TAK-242 (Resatorvid) is another small molecule inhibitor of TLR4 that reduces LPS-induced cytokine secretion and cell death in hepatocytes and monocytes in vitro. Importantly, TAK-242 reduced the severity of inflammation, hepatocyte death and improved organ function in two animal models of ACLF (bile duct ligation + LPS; CCl4 + LPS) and one model of acute liver failure (Galactosamine + LPS) [146]. Monoclonal antibodies have also proven effective, as exemplified by the NI-0101 humanized monoclonal antibody (mAb) that interferes with TLR4 dimerization and activation. In a randomized phase 1 dose escalation study of healthy volunteers receiving LPS, NI-010 was shown to possess durable anti-inflammatory properties, suppressing the production of IL-6, TNFα, CXCL10, and IFNβ [157].

The use of TLR4 antagonists in combination with other treatments, may provide additional therapeutic benefits. Using a murine model of CCl4-induced fibrosis, the TLR4 inhibitor Serelaxin (RLX030), when combined with the PPARγ agonist rosiglitazone, has been shown to amplify the beneficial effects of rosiglitazone and simultaneously reduce hepatic collagen content and HSC activation [158]. In addition, the small molecule Ibudilast, a phosphodiesterase-4 inhibitor, is currently being assessed for the treatment of extrahepatic conditions [159] in combination with other TLR4 antagonists such as TAP2, TLR4-IN-C34 and M62812, which are clinically effective for the management of chronic inflammatory conditions and sepsis. Regardless of how promising TLR4 antagonists are in the treatment of liver fibrosis, there are still challenges in bioavailability and delivery. Nonetheless, anti-TLR4 therapies may represent an alternative strategy for future treatments for liver fibrosis.

(b) Toll-like Receptor 9. TLR9 binds double-stranded CpG unmethylated DNA from bacteria, fungi and viruses, as well as host-derived DNA derived from apoptotic cells. In line with changes observed in intestinal permeability, an increase in circulating bacterial DNA is often an early event in many experimental models of alcoholic liver disease and fatty liver, even preceding hepatic fibrosis [160,161]. Activation of TLR9 from host-derived apoptotic hepatocyte DNA can exacerbate fibrogenic signaling by retaining HSCs at sites of cellular apoptosis, where they become activated and up-regulate collagen production [162]. TLR9 and STING have been shown to synergistically trigger a macrophage pro-inflammatory response to self-mitochondrial DNA (mtDNA) during the development of NASH [163]. This has been confirmed in murine models of diet-induced NASH, where TLR9 deletion or phar-
macological antagonism resulted in an attenuated response to bacterial DNA and mtDNA, leading to reduced IL-1β production, steatosis and liver injury [164]. These findings are supported by TLR9 knockout (KO) models, where mice lacking TLR9 develop less severe steatohepatitis and liver fibrosis when compared to wild-type mice on a choline-deficient l-amino acid-defined diet [165,166].

Compared to TLR4, fewer therapies are available to inhibit TLR9 activation. The novel TLR9 antagonist COV08-0064, a small-molecule inhibitor with greater specificity for TLR9 than oligo-based antagonists, has shown promise in damping hepatocellular death in animal models of sterile liver inflammation [167,168]. A newly developed TLR9 mAb, clone NaR9, has also shown promise, rescuing mice from fulminant hepatitis caused by administering the TLR9 ligand CpGB and D-(+)-galactosamine [169]. Human studies using TLR9 agonism have been exploited in an attempt to improve antiviral responses against chronic infections as well as cancer therapies. TLR9 inhibition, however, is considerably less common, having been employed to limit excessive immune activation in conditions such as IgA nephropathy and Sjogren’s syndrome [167,170,171]. TLR9 antagonism as a treatment for chronic liver disease has not yet been assessed in humans, but warrants attention due to accumulated evidence in animal models and in vitro studies.

(c) Toll-like Receptor 3. Another interesting but often overlooked target in liver inflammatory and fibrotic progression is TLR3. TLR3 activation is generally thought to have a protective and anti-inflammatory role in many models of liver disease. This role was clearly demonstrated in mice fed with a high-fat diet followed by binge drinking to induce liver injury [172]. TLR3 activation by polyinosinic-polycytidylic acid (polyI:C) attenuated liver fibrosis by increasing HSC and KC IL-10 expression, as well as reducing hepatic expression of TNF, IL-6 and CXCL2. TLR3 signaling is well defined in rodent natural killer (NK) cells, where activation of TLR3 results in a potent anti-fibrotic effect [173]. A study by Li et al. showed that hepatic NK cells can be synergistically activated by IL-18 and TLR3 ligand stimulation to induce HSCs apoptosis via TNF-related apoptosis-inducing ligand (TRAIL)-mediated degranulation [174]. On the contrary, exosome-mediated activation of TLR3 in HSCs has been shown to exacerbate liver fibrosis by enhancing IL-17A production by γδ T cells [175].

4.1.2. NLRP3

NLRP3 is an intracellular sensor that detects a broad range of microbial ligands, resulting in activation of the NLRP3 inflammasome. Hepatic NLRP3 inflammasome activation promotes caspase-1-dependent release of the pro-inflammatory cytokines IL-1β and IL-18 by KCs, as well as to gasdermin D-mediated pyroptotic cell death that has been demonstrated in patients with NASH and ASH. NLRP3 inflammasome hyper-activation has been linked to severe hepatic inflammation, fibrosis and hepatocyte pyroptosis in mice [176]. In addition, NLRP3 KO mice, who were fed on a high fat and caloric diet to establish NASH have shown enhanced hepatic MCP-1 expression and extensive M1 macrophage infiltration [177]. PAMP-responsive NLRP3 inflammasome inhibition with either MCC950 or a potent vitamin D receptor agonist, Calcipotriol, can alleviate fibrosis and partially reversed liver scarring in the murine models of NASH and experimental liver cholestasis respectively [178,179]. In humans, other investigational NLRP3 inhibitors such as NT-0167 and IFM-2427, which have demonstrated reduction in IL-1β and IL-18 in preclinical trials, are still being evaluated in a phase 1 clinical trial for treatment of numerous chronic inflammatory and fibrotic diseases [180,181].

4.2. Targeting Liver Macrophages

Hepatic macrophages hold a central position in maintaining homeostasis in the liver as well as in the pathogenesis of disease. Evidence suggests that KCs release CCL2 (C-C motif chemokine ligand 2) in response to acute insult to recruit pro-inflammatory and profibrogenic monocytes. KCs also possess a fibrogenic role during chronic liver disease by activating HSCs via secretion of TGFβ, platelet-derived growth factor (PDGF) and connective tissue
growth factor (CTGF/CCN2). A profibrogenic niche is established in the liver via additional production of pro-inflammatory cytokines (TNF, IL-1β, IL-6) and chemokines (CCL1, CCL2 and CCL5) that exacerbate chronic inflammation and fibrosis progression. Activated HSCs undergo a phenotypic switch from a quiescent into proliferative, myofibroblast-like cells, exhibiting upregulated collagen synthesis, increased proliferation, migration and a relative resistance to apoptosis [182]. On the other hand, KCs also promote resolution of fibrosis and degradation of extracellular matrix via induction of metalloproteinases (MMP-9, -12 and -13) [35]. Given this role as first-line responders to liver injury and their dual functions in liver disease, hepatic macrophages are, in principle, intriguing therapeutic targets (Figure 4). Notwithstanding, macrophages exert a broad range of functions in the liver and consist of heterogeneous cellular subsets, thus rendering the development of macrophage-based interventional strategies targeting hepatic fibrosis a challenging task.

**Figure 4.** Potential targets of hepatic macrophages to treat liver fibrosis. 1—Inhibition of inflammatory monocyte/macrophage recruitment through the inhibition of CCL2 (MCP-1) by RNA-based silencing (e.g., RNA-aptamer mNOX-E36), small molecule or monoclonal antibody (mAb). 2—Shaping and polarization of hepatic macrophage via biological engineering using nanoparticles or targeting drugs (e.g., dexamethasone [DEX] liposomes/microbubbles) from the pro-inflammatory to regenerative phenotype. 3—Restoration of hepatic macrophage count and function using IL-4 and CX3CL1 [183], macrophage colony stimulating factor (M-CSF) inhibitors or autologous macrophage-based cell therapy. CCL5/CCR5, C-C Chemokine ligand 5/C-C chemokine receptor type 5; CD206, Mannose receptor; HALPC, human allogeneic liver-derived progenitor cells; HepaStem® is an allogenic stem cell therapy product using human adult liver-derived progenitor cells; M2, macrophage type 2; G-CSF, granulocyte colony-stimulating factor; PPARs, peroxisome proliferator-activated receptors.

### 4.2.1. Inhibition of Inflammatory Monocyte Recruitment

The infiltration of inflammatory monocytes into the liver is critically regulated by the chemoattractant properties of several chemokines. The chemokine CCL2 and its receptor CCR2 represent the critical trigger for acute monocyte infiltration into the liver in alcoholic liver disease, NAFLD/NASH, and viral hepatitis, among others, representing a key initial...
step in the fibrosis that ensues [184]. During the early phase of liver injury in CCl₄ induced liver injury model, inflammatory Ly 6C<sup>high</sup> monocytes directly activate HSCs in a TGF-β-dependent manner, with CCL2 playing a central role in their recruitment [185]. Indeed, targeted removal of infiltrated monocytes during fibrosis development using CD11b-diphtheria toxin receptor transgenic mice, reduced fibrosis in the CCl₄ liver injury mouse model. Importantly, the depletion of these macrophages during resolution phase did not hamper HSC activation and ECM deposition, supporting the role of infiltrating monocytes in fibrosis initiation [186].

Due to the key role played by CCL2 in fibrosis initiation, interventions targeting CCL2-mediated recruitment are an attractive strategy to limit fibrosis initiation and progression. The CCL2 neutralizing RNA-aptamer mNOX-E36 has proven effective in ameliorating steatosis development and fibrosis progression in CCl₄ or MCD models by preventing inflammatory monocyte recruitment into the liver [187]. Blockade of the CCL2 receptor CCR2 with the selective inhibitor Propagermanium has also been shown to reduce disease activity in a mouse model of NASH by reducing macrovesicular steatosis and lobular inflammation at early stages of disease [188].

The dual CCR2/CCR5 inhibitor, Cenicriviroc (CVC), has also been shown to effectively hamper CCL2-mediated monocyte recruitment and to exert antifibrotic effects in mouse models of ALD and NASH [189–191]. Despite promising results and success in early trials for treatment of liver fibrosis in NASH patients where improvement in fibrosis by ≥1 stage was achieved after 1 year of CVC (NASH CRN system) [192,193], the phase 3 clinical trial (AURORA) was discontinued due to lack of efficacy. (Tobira Therapeutics, Inc., NCT03028740) [194].

Maraviroc (Selzentry/Celsentri) is a sole CCL5 inhibitor that has been shown to reduce hepatic fibrosis progression and improve disease in murine NAFLD/NASH models [195]. Currently, Maraviroc is being evaluated in Phase 4 open-label study in combination with antiretroviral therapy in people living with HIV-1 as a treatment for NAFLD [196] (Brighton and Sussex University Hospitals NHS Trust, 2017-004141-24).

4.2.2. Shape and Polarization of Hepatic Macrophage Function

A promising strategy for treatment of liver fibrosis is via modulation of the functional switch between pro-inflammatory and regenerative liver macrophages. This approach uses bioengineered nanoparticles or “polarizing” drugs tailored to induce selective and functional reprogramming of hepatic macrophages [197]. The advantage of this approach is the absence of off-target effects when compared with conventional systemic therapies. This is mediated by the innate scavenging activity of KCs that drives the uptake of most nanomaterials and drug delivery systems in the liver. The addition of mannose or other carbohydrate-functionalyzed polymers to the surface of nanoparticles can potentially further improve delivery of anti-inflammatory drugs to hepatic macrophages due to their high expression of mannose receptors such as CD206. Other drug delivery systems, such as liposomes and microbubbles can be loaded with anti-inflammatory treatments such as corticosteroids (dexamethasone (DEX)), IL-4, IL-10 and PGE2, and have additionally been proposed/ utilized as potential macrophage-specific treatment approaches in murine models [198].

The delivery of DEX-loaded vehicles has demonstrated efficacy in attenuating liver fibrosis in a murine model of CCl₄ liver injury. Intravenous injections of DEX liposomes resulted in a significant reduction in fibrosis, inhibition of T-cell accumulation in liver and functional skewing of liver macrophages toward an M2 phenotype [199]. Modifying the surface chemistry of nanoparticle drug carriers can also induce immunomodulatory effects on hepatic macrophages [200]. Liposome-encapsulated lipophilic curcumin or 1,25-dihydroxy-vitamin D₃ (calcitriol), when injected intravenously into mice with diet-induced NASH, shifted the hepatic dendritic cell inflammatory profile towards a regulatory phenotype, reduced inflammation and suppressed immune activation and collagen deposition [201].
Galectin-3 (Gal-3) is a $\beta$-galactoside-binding lectin expressed primarily in macrophages that stimulates TGF-$\beta$ production, myofibroblast activation and extracellular matrix production. The galectin-3 inhibitor GR-MD-02 (Belapectin) has been shown to reduce hepatic fibrosis and portal hypertension when used in murine models of cirrhosis [202,203]. Unfortunately, in a phase 2b trial of 162 patients with NASH, cirrhosis, and portal hypertension, Belapectin had no significant effect on HVPG and fibrosis but proved effective at reducing the risk of variceal developments in selected patients with NASH-induced cirrhosis (Galectin Therapeutics Inc., NCT02462967) [204].

A family of therapeutic targets that has gained attention over recent years is the peroxisome proliferator-activated receptors (PPAR$\alpha$, $\beta/\delta$, and $\gamma$); a group of nuclear transcription factors differentially expressed among hepatic cell types including macrophages. Dysregulated PPARs during chronic hepatic injuries contribute to liver disease progression and major metabolic dysfunctions. Moreover, beneficial effects from activating one or multiple PPAR isoforms on reversing fibrosis, as well as phenotypic enhancement of different liver cell types, have been observed in preclinical studies. Thus, strategies that modulate PPAR activity have the potential to induce macrophage polarization. Activation of PPAR-$\gamma$ in particular has emerged as a key regulator of hepatic macrophage polarization, stimulating an anti-inflammatory M2 phenotype [205].

In vitro administration of the pan-PPAR agonist Lanifibranor has been shown to significantly reduce portal pressure, improve intrahepatic vascular resistance and prevent ascites in the rodent model of TAA or common bile duct ligation induced cirrhosis. The underlying mechanisms of the hemodynamic ameliorations involve marked deactivation of HSCs and inhibition of inflammatory macrophages probably via PPAR$\delta$ agonism [206–208]. In humans, Lanifibranor exerted positive metabolic and anti-fibrotic effects in adult patients with NASH [208]. In a short 24-week clinical trial (NATIVE study), a single dose of Lanifibranor when given daily significantly decreased liver inflammation and prevented worsening of fibrosis in 49% of participants compared with 27% in the placebo arm [209,210]. Currently, Lanifibranor is being investigated in a phase 3 study of adults with NASH with stage 2/3 liver fibrosis (Inventia Pharma NCT04849728). Similarly, Pioglitazone and Saroglitazar, selective PPAR-$\gamma$ and PPAR-$\alpha$ agonists, have been shown to improve NASH [211]. Pioglitazone reduced NAS by at least 2 points without worsening of fibrosis in more than half of patients [212] (University of Florida, NCT00994682). Likewise, Saroglitazar significantly improved liver stiffness measurement on FibroScan in NAFLD patients with diabetic dyslipidemia [213] (Zydus therapeutics Inc., NCT03061721).

4.2.3. Restoration of Hepatic Macrophage Count and Function

Macrophage polarization is a continuum of overlapping functional states that involve a plethora of signals and corresponding dynamic gene expression programs. During fibrogenesis, inflammatory monocytes are recruited to the inflamed liver, forming profibrotic macrophages. These cells phagocytose cellular debris, activating the ERK signaling cascade and forming restorative macrophages that orchestrate fibrinolysis. In line with this observation, it is speculated that phagocytosis elicits significant effects on macrophage phenotype and function to promote restorative differentiation pathways [214,215]. Therefore, induced phagocytic behavior in vivo has potential as a translational strategy for the treatment of hepatic fibrosis.

Another therapeutic intervention consists of autologous macrophage-based cell therapy, which entails ex vivo culturing of peripheral blood mononuclear cells under selective conditions to induce antifibrotic or fibrinolytic subsets. Following differentiation, macrophages are intravenously infused back into patients to hypothetically improve fibrosis. A first-in-human, phase 1, single-arm, dose escalation clinical trial in nine patients with compensated liver cirrhosis showed that administration of macrophages was safe, with no clinically relevant adverse reactions recorded during the infusion or in the immediate post-infusion period. Several non-invasive measures of liver fibrosis were improved following macrophage infusion, including transient elastography, serum-enhanced liver fibrosis
score and the collagen turnover markers PRO-C3 and C3M, highlighting the potential antifibrotic effect of autologous monocyte-derived macrophage infusion in cirrhosis [216] (ISRCTN 10368050).

Another potential strategy utilizes bone marrow-derived stem cell (BMSC) engraftment onto the damaged liver, by using cytokines such as macrophage colony-stimulating factor (M-CSF) or granulocyte colony-stimulating factor (G-CSF) in advanced liver cirrhosis [217]. Both cytokines are hematopoietic growth factors that affect monocytopoiesis and monocyte release from bone marrow. In a single-center randomized trial of 50 patients with decompensated cirrhosis, a combination of G-CSF and erythropoietin (Darbepoetin α) improved survival by 1 additional year as compared to the placebo group [218] (Institute of Liver and Biliary Sciences, India, NCT01384565).

Cell-based therapies such as infusion of bona fide allogeneic liver-derived progenitors is another attractive strategy to dampen the pro-inflammatory hepatic milieu, inhibit HSC activation and fibrogenesis. HepaStem® is advertised as a highly advanced cell therapy platform consisting of human liver-derived stem cells that are ethically obtained from healthy donors and expanded in a current good manufacturing practice (cGMP)-compliant environment [219]. These progenitors have been tested in a phase 2a study in NASH, and a phase 2a study in ACLF, the first ever study to use stem cells to treat such indications. The results show that human allogeneic liver-derived progenitor cells (HALPC) infusion reduce systemic inflammatory markers and decrease altered liver function in surviving ACLF patients. The 28-day and 3-month survival rates were 83% and 71%, and no patient had ACLF at 3 months [220] (Promethera Biosciences (NCT04229901), EudraCT 2016-001177-32).

5. Conclusions

In light of the changing nature of CLD epidemiology, there is an ever-growing demand for novel targets and specific treatments that reverse or cease fibrosis progression. Interactions between the gut microbiome, the immune system and the liver are increasingly implicated in CLD progression and severity. Moreover, the steady advances over the last decade in basic research exploring mechanisms of hepatic fibrosis coupled with thorough dissection of the intricate gut–liver relationship have spurred promising avenues for identification of strategies to prevent liver fibrosis. Several of these strategies have already fueled a robust therapeutic pipeline across a variety of novel targets. Many of these emerging therapeutics have been systematized to target the intestinal mucosa, intestinal microbiome, or hepatic immune response. Additionally, some of these emerging therapies have shown beneficial effects on portal pressure and extrahepatic benefits such as improvement of body weight, lipid profile, glycemic control and others. Strategies involving combination therapies with different antifibrotic agents or monotherapy with multi-target drugs may be more effective given the complex mechanisms and pathways involved in hepatic fibrosis. However, standardized, stringent and unbiased interventional trials are still required for successful translation of any strategy into real world clinical practice.

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# List of Abbreviations

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>AASLD</td>
<td>American Association for the Study of Liver Diseases</td>
</tr>
<tr>
<td>ACLF</td>
<td>Acute-on-chronic liver failure</td>
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<tr>
<td>ALD</td>
<td>Alcoholic liver disease</td>
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<tr>
<td>ASH</td>
<td>Alcoholic steatohepatitis</td>
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<tr>
<td>BAs</td>
<td>Bile acids</td>
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<tr>
<td>BT</td>
<td>Bacterial translocation</td>
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<tr>
<td>CCL2/MCP-1</td>
<td>Chemokine (C-C motif) ligand 2/ Monocyte chemoattractant protein-1</td>
</tr>
<tr>
<td>CCL5</td>
<td>Chemokine (C-C motif) ligand 5</td>
</tr>
<tr>
<td>CCl₄</td>
<td>Carbon tetrachloride</td>
</tr>
<tr>
<td>CCR5</td>
<td>C-C chemokine receptor type 5</td>
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<tr>
<td>CLD</td>
<td>Chronic liver disease</td>
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<tr>
<td>CTGF/CCN2</td>
<td>Connective tissue growth factor</td>
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<td>CVC</td>
<td>Cenicriviroc</td>
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<tr>
<td>DAMPs</td>
<td>Damage-associated molecular patterns</td>
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<tr>
<td>DMR</td>
<td>Duodenal mucosal resurfacing</td>
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<tr>
<td>DEX</td>
<td>Dexamethasone</td>
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
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<tr>
<td>ESAL</td>
<td>European Association for the Study of the Liver</td>
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<tr>
<td>FGF19</td>
<td>Fibroblast growth factor 19</td>
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<td>FGF21</td>
<td>Fibroblast growth factor 21</td>
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<td>FMT</td>
<td>Fecal microbiota transplantation</td>
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<td>FXR</td>
<td>Farnesoid X receptor</td>
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<td>Gal-3</td>
<td>Galectin-3</td>
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<tr>
<td>G-CSF</td>
<td>Granulocyte colony-stimulating factor</td>
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<tr>
<td>GIP</td>
<td>Glucose-dependent insulinotropic peptide</td>
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<tr>
<td>GLP1</td>
<td>Glucagon like peptide-1</td>
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<td>GLP-1R</td>
<td>Glucagon-like peptide-1 receptor</td>
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<td>HE</td>
<td>Hepatic encephalopathy</td>
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<tr>
<td>HMGB1</td>
<td>High-mobility group box 1</td>
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<td>HSCs</td>
<td>Hepatic stellate cells</td>
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<td>HVPD</td>
<td>Hepatic venous pressure gradient</td>
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<td>IL-1β</td>
<td>Interleukin one beta</td>
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<td>Interleukin six</td>
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<tr>
<td>ISAPP</td>
<td>International Scientific Association for Probiotics and Prebiotics</td>
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<tr>
<td>KCs</td>
<td>Kupffer cells</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>LBP</td>
<td>Lipopolysaccharide binding protein</td>
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<tr>
<td>LSECs</td>
<td>Liver sinusoidal endothelial cells</td>
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<tr>
<td>mAb</td>
<td>Monoclonal antibody</td>
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<tr>
<td>MAFLD</td>
<td>Metabolic associated fatty liver disease</td>
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<tr>
<td>MAMPs</td>
<td>Microbial -associated molecular patterns</td>
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<tr>
<td>M-CSF</td>
<td>Macrophage-colony stimulating factor</td>
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<tr>
<td>MCD</td>
<td>Methionine Choline-deficient</td>
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<tr>
<td>MELD</td>
<td>Model for end-stage liver disease</td>
</tr>
<tr>
<td>mtDNA</td>
<td>Mitochondrial DNA</td>
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<tr>
<td>NAS</td>
<td>NAFLD activity score</td>
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<td>NASH</td>
<td>Non-alcoholic steatohepatitis</td>
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<tr>
<td>NF-κB</td>
<td>Nuclear factor-kappa B</td>
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<tr>
<td>NK</td>
<td>Natural killer</td>
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<tr>
<td>NSBBs</td>
<td>Nonselective beta blockers</td>
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<tr>
<td>OCA</td>
<td>Obeticholic acid</td>
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<tr>
<td>PAMPs</td>
<td>Pathogen-associated molecular patterns</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
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<tr>
<td>PPARs</td>
<td>Peroxisome proliferator-activated receptors</td>
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<tr>
<td>PRRs</td>
<td>Pattern recognition receptors</td>
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</tbody>
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PTH  Portal hypertension
RCT  Randomized control trial
RIG-1  Retinoic acid-inducible gene I
ROS  Reactive oxygen species
SBP  Spontaneous bacterial peritonitis
SCFA(s)  Short-chain fatty acids
SSD  Soluble solid dispersion
STING  Stimulator of interferon genes
TAA  Thioacetamide
TGF-β  Transforming growth factor beta
TGR5  Takeda G-protein-coupled receptor 5
TLRs  Toll-like receptors
TMA  Trimethylamine
TMAO  Trimethylamine-N-oxide
TNF-α  Tumor necrosis factor alpha
Uro A  Urolithin A
VH  Variceal hemorrhage
ZO-1  Zonula occludens-1
α-SMA  Alpha Smooth muscle actin

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