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THEMED ISSUE REVIEW

Chronic liver diseases: From development to novel pharmacological therapies: IUPHAR Review 37

Maria Teresa Borrello | Derek Mann

Newcastle Fibrosis Research Group, Biosciences Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK

Correspondence
Derek Mann, Newcastle Fibrosis Research Group, Biosciences Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK.
Email: derek.mann@ncl.ac.uk

[Correction added on 29 April 2022, after first online publication: Figures 1 and 3 images have been interchanged in this version.]

Chronic liver diseases comprise a broad spectrum of burdensome diseases that still lack effective pharmacological therapies. Our research group focuses on fibrosis, which is a major precursor of liver cirrhosis. Fibrosis consists in a progressive disturbance of liver sinusoidal architecture characterised by connective tissue deposition as a reparative response to tissue injury. Multifactorial events and several types of cells participate in fibrosis initiation and progression, and the process still needs to be completely understood. The development of experimental models of liver fibrosis alongside the identification of critical factors progressing fibrosis to cirrhosis will facilitate the development of more effective therapeutic approaches for such condition. This review provides an overlook of the main process leading to hepatic fibrosis and therapeutic approaches that have emerged from a deep knowledge of the molecular regulation of fibrogenesis in the liver.

KEYWORDS
chronic liver diseases, fibrosis, fibrosis therapy, macrophages, pharmacology of chronic liver diseases

1 | CIRRHOSIS AND CHRONIC LIVER DISEASE

Deaths from cirrhosis worldwide doubled in the period between 1990 and 2017, rising to in excess of 2 million early fatalities (Sepanlou et al., 2020). In addition, during this period the major cancer associated with cirrhosis, hepatocellular carcinoma (HCC), has become the fourth major cause of cancer-related deaths worldwide (Yang et al., 2019). The majority of aetiologies of chronic liver disease (that lead to cirrhosis are, at least in theory, either preventable or can now be effectively treated. However, there remain significant barriers to lowering the incidence of chronic liver disease of which the most challenging is the effective implementation of national and international polices for the prevention and treatment of liver damage. Furthermore, for the majority of affected people, chronic liver disease is indolent and only manifests to a symptomatic state at the point where the architecture of the liver is sufficiently damaged to impact on the normal functions of the liver, or when a cancer develops. Hence, the majority of early stage chronic liver disease is undetected until it presents in an advanced state at which point for many patients an effective cure is often limited to organ transplantation (Yang et al., 2019).

Chronic liver disease is described as a chronic inflammatory condition of the liver (lasting longer than 6 months) that leads to destruction and impaired regeneration of the liver parenchyma, leading ultimately to fibrosis and cirrhosis (Quaglia et al., 2016). The major causes of chronic liver disease are non-alcoholic fatty liver disease (NAFLD; Golabi et al., 2016), alcohol-related liver disease, viral hepatitis, various inherited metabolic conditions and autoimmune liver diseases. In addition, around 15% of cases of chronic liver disease are idiopathic (Moon et al., 2020). Despite alcohol-related liver disease

Abbreviations: ECM, extracellular matric; KLF, Krupple-like transcription factor; MoMϕs, monocyte-derived macrophages; MSC, mesenchymal stem cells; TREM2, triggering receptor expressed on myeloid cells 2; UPR, unfolded protein response.

remaining the non-viral chronic liver disease associated with the highest numbers of cirrhosis deaths, non-alcoholic fatty liver disease is now the global imperative to solve, as it is the fastest growing cause of chronic liver disease and is estimated to have a prevalence of 25% in the global population rising to 95% in the morbidly obese (Arshad et al., 2020). Non-alcoholic fatty liver disease begins as a benign condition but, in a significant proportion of individuals, it can develop into non-alcoholic steatohepatitis (NASH) (Dam-Larsen et al., 2004; Tsukuma et al., 1993). Of all the different histological characteristic of non-alcoholic steatohepatitis, which include steatosis, hepatocyte ballooning and death, and lobular inflammation, the best predictor of the patient outcome is the degree of fibrosis. Younessi et al. (2019) documented the evolution of non-alcoholic fatty liver disease and demonstrated a clear correlation between the degree of fibrosis progression and patient outcome, including ultimately the development of hepatocellular carcinoma (Mazzarelli et al., 2020). As fibrosis is common to the pathobiology of the majority of aetiologies of chronic liver disease. It is now recognised as a dynamic process with the potential to either progress or resolve and it is a very attractive therapeutic target for the prevention and treatment of cirrhosis. In this review, we will examine the biology of liver fibrosis and the current therapeutic landscape and consider the major unanswered questions for which answers will unlock future therapeutic opportunities.

2 | THE PATHOBIOLOGY OF LIVER FIBROSIS

2.1 | The fibrotic matrix

Cirrhosis represents the evolution of chronic liver disease to the point at which liver architecture is severely disrupted, characteristically featuring the appearance of numerous regenerative hepatocellular nodules and extensive vascular reorganisation, the latter arising from neo-angiogenesis (DeLeve et al., 2004; Moon et al., 2020). Underlying these architectural changes is the fibrotic process which involves the net deposition of collagen-rich fibril-forming extracellular matrix (ECM) that forms a scar-like tissue. The anatomical localisation of the scar accumulation is characteristic of the aetiology of the parenchymal injury:

- Portal-based fibrosis develops in chronic hepatitis, chronic cholestasis and haemochromatosis.
- Central-based fibrosis develops in steatotic liver diseases of alcoholic or non-alcoholic aetiologies and in venous outflow obstruction.

With the progression of fibrosis, so-called fibrous septa are formed and can mature to link vascular structures. Alongside these anatomical changes are qualitative and quantitative modifications to the ECM components of the sinusoidal/perisinusoidal liver structures. In particular, collagens I and III and non-collagenous ECM proteins such as laminin and fibronectin are deposited into the space of Disse. These ECM deposits obstruct the exchange of key nutrients and metabolites between hepatocytes and the sinusoidal blood (the defenestration process) (Arriazu et al., 2014; Karsdal et al., 2015). When the parenchyma is injured, hepatic stellate cells are transformed to myofibroblasts producing ECM constituents, while in the portal tracts, resident myofibroblasts play important role with the activation of cholangiocytes to matrix-producing cells, possibly contributing to portal/periportal fibrosis (Lepreux & Desmoulière, 2015).

As liver disease perpetuates, more pronounced architectural changes due to fibrosis and neo-angiogenesis occur that result in the development of cirrhosis. Cirrhosis presents as a diffuse phenomenon where bridging fibrous septa completely circumscribe structurally abnormal nodular areas of regenerating liver parenchyma. The pathophysiologic consequences of cirrhosis mainly depend on the severity of the associated vascular changes (Schuppan & Afdhal, 2008).

Based on the size of the parenchymal nodules, cirrhosis has been classified as:

- micronodular (nodules <3 mm),
- macronodular (nodules >3 mm), and
- mixed (nodules of variable sizes)

Therefore, progressive chronic liver disease represents a perpetuated process of hepatic fibrogenesis, liver tissue architectural distortion and remodelling. Importantly, fibrosis was once thought to be unidirectional. It is now acknowledged to be highly dynamic, such that the progression to cirrhosis is determined by the balance between fibrogenesis and fibrolysis, the latter being the natural process of breakdown of fibrotic ECM (Adams, 2011). Moreover, tipping the balance towards fibrolysis can promote regression of established fibrosis, which is highly encouraging for developing strategies aimed at reversing advanced liver fibrosis (Gieling et al., 2008). The factors determining this dynamic balance are highly complex, but at the level of the ECM are at least in part controlled by activities of collagen-degrading **matrix metalloproteinases (MMP)**. In the diseased liver there is high-level over-expression of the **metallopeptidase inhibitor 1 (TIMP1)**, which has a broad inhibitory effect on collagen-degrading MMP) Karsdal et al., 2015; Lee & Friedman, 2011). Indeed, serum levels of TIMP1 are also highly elevated in chronic liver disease and can be used alongside other surrogate markers of fibrosis for minimal invasive detection and grading of liver fibrosis (Parkes et al., 2011; Xie et al., 2014). Experimental studies have reported that transgenic over-expression of *Timp1* promotes liver fibrosis and delays the rate at which fibrosis resolves (Yoshiji et al., 2000, 2002), while a *TIMP1* antagonist has been shown to be anti-fibrotic in a model of pre-established liver fibrosis (Parsons et al., 2004). However, the absolute requirement for TIMP1 for fibrogenesis is challenged by more recent studies with *Timp1* knockout mice, in which the absence of the MMP inhibitor appeared to not prevent the development of fibrosis induced by either bile duct ligation or carbon tetrachloride (CCl_4)-induced liver injury (Thiele et al., 2017). Under these latter conditions, it is likely that other MMP inhibitors compensate for the lack of *Timp1*. A further contributory factor in the degree to which fibrotic ECM can

be remodelled, is its degree of maturation, in particular the extent of covalent cross-linking between collagen and elastin molecules in the ECM can determine the potential for reversibility of fibrosis (Chen et al., 2020). The **lysyl oxidase family** (**LOX** and LOXL1-4) are important in this regard as they are ECM cross-linking enzymes that are increasingly up-regulated as fibrosis progresses to cirrhosis (Chen et al., 2020). **LOXL2** was the first member of the family to be therapeutically targeted thanks to the development of the humanised monoclonal antibody **simtuzumab** (Schuppan et al., 2018). Unfortunately, this approach failed to achieve clinical efficacy in phase 2 trials examining efficacy towards liver fibrosis or fibrosis in other organ systems (Vuppalanchi et al., 2021).

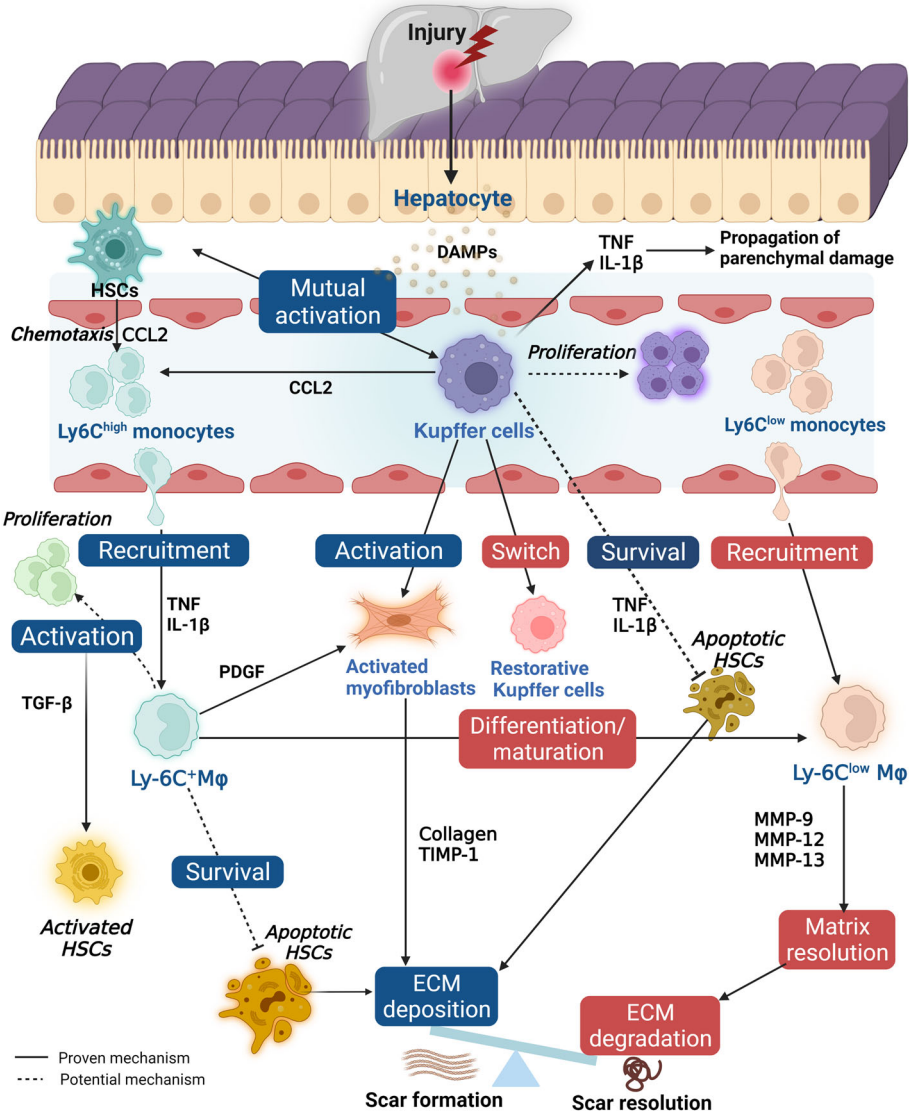

FIGURE 1 Activation of macrophages after liver injury. Following hepatic injury, the hepatic epithelial cell release danger associated molecular pattern molecules (DAMPS), consisting in RNA, DNA and alarmines. These molecules activate the resident Kupffer cells in the space of Disse. The Kupffer cell releases inflammatory cytokines (**IL-1β** and TNF) propagating the parenchymal damage. Kupffer cell activation promotes the hepatic stellate cells activation and TGFβ1 sensitisation, secretion of CCL3 and potent activation of Kupffer cells. Increment in CCL2 favours chemotaxis of bone marrow derived classical Ly6C^{high} monocytes. These enter the liver and develop in infiltrating Ly6C^{+} macrophages promoting the secretion of inflammatory cytokines. Newly infiltrating hepatic macrophages booster the increment of chronic liver disease (CLD), and fibrosis through TGFβ-PDGF mediates hepatic stellate cells proliferation and transdifferentiation. Altogether, these signalling pathways promote the accumulation of collagen forming scar tissue. However, the contribution of locally proliferating hepatic macrophages to the initiation and progression of chronic liver injury remains debated (dashed lines). Following liver injury Kupffer cell can switch to restorative Kupffer cells, promoting extracellular matrix (ECM) degradation and fibrosis resolution. Also, Ly6Chi macrophages can adopt a restorative phenotype, characterised by Ly6C^{low} expression and the capacity to degrade excessive ECM via secretion of metalloproteinases such as MMP-9-12-13 and to induce ultimately hepatic stellate cell apoptosis. Increase of apoptotic particle can further boost macrophage restorative phenotype. The function of newly recruited Ly6C^{low} remains elusive. Globally, these paths lead to ECM degradation during liver fibrosis

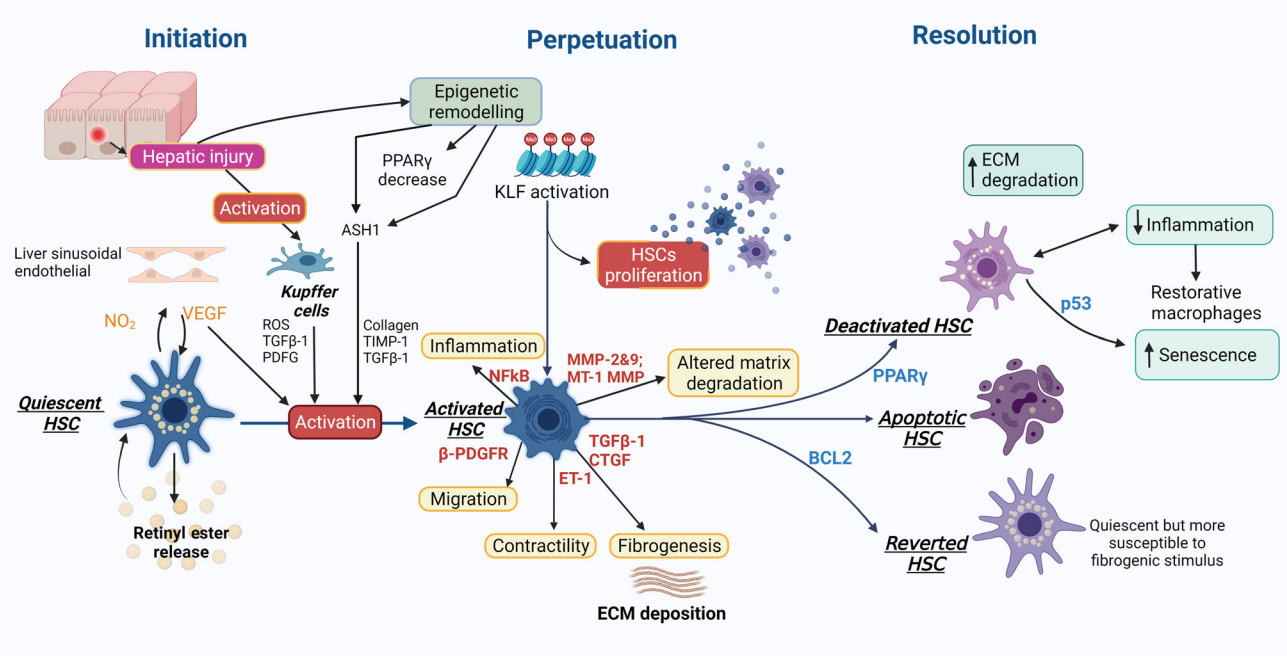

FIGURE 2 Activation of hepatic stellate cells (HSCs) after liver injury. During normal liver homeostasis, the unactive stellate cells serve as storage for retinyl ester and participate in liver regeneration by secreting mitogens and other signals to hepatocytes. It also participates in a bidirectional cross-talk with liver sinusoidal endothelial cells (LSECs). After liver injury, HSCs transdifferentiate into myofibroblast-like cells that represent the main collagen-producing cell in the liver (activated stellate cells). Finally, as the liver injury subside, activated stellate cells are removed from the liver through different process. The apoptotic process is tightly regulated whereas deactivation is quite unknown process; during deactivation, cells become inactive, and they start to senesce. There is a decrease of inflammatory cytokines in the microenvironment and increase of restorative macrophages and an increase in extracellular matrix (ECM) degradation. An even more unknown process in the reversion of HSCs where cells become quiescent but more sensitive to fibrogenic stimuli

2.2 | The major cellular players

The liver is one of the biggest organs in the body, performing both endocrine and exocrine functions. Sixty to seventy percent of the total cell population of the liver is made up from hepatocytes with the remaining cellular mass being cholangiocytes, non-parenchymal cells including Kupffer cells, hepatic stellate cells, liver sinusoidal endothelial cells and liver-associated lymphocytes (Williams & Iatropoulos, 2002). Following liver injury and depending on whether damage is resolved or not, there are profound quantitative and qualitative changes in these cellular constituents that help dictate the progression of fibrosis (Figures 1 and 2).

2.2.1 | Hepatocytes and cholangiocytes

The initial response to liver injury is hepatocellular stress and cell death. Injured hepatocytes release **reactive oxygen species (ROS)** and **damage-associated molecular patterns (DAMPs)** that can function as fibrogenic mediators (Figure 1). Damaged and dying hepatocytes are a major stimulus for triggering Kupffer cell and hepatic stellate cell activation, and for enhancing the pro-fibrotic activity of myofibroblasts (Bataller & Brenner, 2005; Schattenberg et al., 2012). Hepatic injury is often associated with hepatocyte apoptosis, which can promote inflammation, fibrogenesis and ultimately cirrhosis (Guicciardi et al., 2013; Guicciardi & Gores, 2010). The apoptotic process can occur via death receptor-mediated (death receptors such as Fas (CD95), TNF-α-receptor 1 (TNF-R1) and death receptor 4 and 5) or mitochondrial mediated pathways and is responsible for inflammation associated with viral hepatitis, alcoholic and fatty liver diseases and cholestatic disorders (Guicciardi et al., 2013). Prevention of hepatocyte apoptosis has been examined as a potential therapeutic approach for liver disease. However, despite its initial promise, the pan-**caspase** inhibitor **emricasan** failed to demonstrate improvements for inflammation or fibrosis in a recently reported Phase 2 trial in non-alcoholic steatohepatitis (Drenth & Schattenberg, 2020). Although apoptosis is the most common pathway of hepatocytic cell death, necrosis and necroptosis also occur (Chowdhury et al., 2009; Ding & Wang, 2014; Guicciardi et al., 2013). Necrosis is characterised by swelling of cells and organelles that causes blebs. This prompts organelle and membrane rupture and leaking of intracellular contents into the extracellular environment, promoting an extensive immunogenic and fibrogenic response (Krishna, 2017; Nanji & Hiller-Sturmhöfel, 1997). Mitochondrial permeability dysfunction, disruption of calcium homeostasis and ADP^{3-} loss promote cell swelling (Soustiel & Zaaroor, 2012). Hepatocyte necrosis is also associated with carbon tetrachloride induced injury, which is a widely employed model for studying liver fibrosis in rodents (Ding & Wang, 2014). Necrosis is a potent suppressant of the hepatic regenerative response and, as it is

often, dependant on the intensity and duration of the stimuli. Necrosis can be widespread and could lead to acute liver failure (Michalopoulos & DeFrances, 1997). Necroptosis is a relatively newly discovered pathway for controlled necrotic cell death mediated by the **receptor-interacting protein serine/threonine kinase 1 (RIPK1) and 3 (RIPK3)** (Schwabe & Luedde, 2018). Necroptosis promotes acute liver inflammation and is associated with **paracetamol** and alcohol-derived hepatic injury. Of note, RIPK3-dependent necroptosis is implicated in fibrosis progression and mice lacking this mode of cell death produce less fibrosis in response to carbon tetrachloride and thioacetamide-induced liver injuries (Jia et al., 2018).

A feature of the aged and/or chronically damaged liver is hepatocyte telomere shortening and replicative senescence, with evidence that the length of telomeres is inversely correlated with severity of fibrosis/cirrhosis (Ferreira-Gonzalez et al., 2021). This association between hepatocellular senescence and fibrosis has also been noted in experimental models either where telomerase is deleted, which accelerates fibrosis, or when the p21 cell cycle arrest gene is deleted which protects from fibrosis (Rudolph et al., 2000; Yosef et al., 2017). Hepatocyte senescence not only limits the regenerative capacity of the liver (Bird et al., 2018) but can also promotes inflammation and fibrosis through the senescence-associated secretory phenotype (SASP), which comprises a plethora of cytokines, chemokines, angiogenic factors and profibrogenic molecules including **transforming growth factor beta-1 (TGFβ1)** and **platelet derived growth factor (PDGF)** which are powerful stimulators of hepatic stellate cell activation (Basisty et al., 2020). In addition, the senescence-associated secretory phenotype recruits inflammatory cells such as neutrophils which can in turn promote the spread of senescence and are implicated in the development of steatosis (Lagnado et al., 2021; Ogrodnik et al., 2017). As such, strategies that manipulate senescence may be of therapeutic interest. However, as senescence protects from carcinogenesis, this concept is not without risk in the context of chronic liver disease, where the risk of developing a primary liver cancer is elevated.

Cholangiocytes are epithelial cells that are critical in the production and transport of bile and its constituents (Banales et al., 2019). Injuries, infections and cholangiopathies are associated with the activation of cholangiocytes which is associated with proliferation and secretion of pro-inflammatory and profibrogenic soluble mediators (Fabris et al., 2019; Sirica et al., 2019). Cholangiocyte-mesenchymal inflammatory cross-talk can promote the ductular reaction which is characterised by bile duct proliferation and is commonly observed in primary biliary cholangitis (PBC) and primary sclerosing cholangitis, both of which are associated with progressive fibrosis (Sato et al., 2019). The ductular reaction has been associated with hepatocyte senescence and hepatic stellate cell activation as potential sources of cholangioacyte activation.

2.2.2 | Liver sinusoidal endothelial cells (LSECs)

Liver sinusoidal endothelial cells comprise 50% of non-parenchymal cells of the liver and form the endothelial lining of the hepatic sinusoids. Functions for liver sinusoidal endothelial cells in the healthy liver include their role as regulators of bidirectional transport of macromolecules between the blood and epithelial liver cells, removal of cell debris and elimination of immune complexes, and they have a variety of immunomodulatory activities (Ma et al., 2021). In response to liver damage, liver sinusoidal endothelial cells undergo phenotypic and structural changes of which the most well documented is capillarisation involving loss of fenestrae, which affects their transport activities. Importantly, loss of the specialised phenotype of liver sinusoidal endothelial cells which accompanies defenestration precedes the initiation of fibrosis and appears to be pivotal as the fully differentiated liver sinusoidal endothelial cell phenotype promotes the quiescent non-fibrogenic phenotype of the hepatic stellate cell (Xie et al., 2012). Capillarisation results in hypoxia, which can stimulate angiogenesis, the latter is intimately linked to fibrogenesis (Ehling et al., 2014). Following capillarisation liver sinusoidal endothelial cells can stimulate fibrogenesis via their release of exosomes, in particular the presence of **sphingosine kinase-1(SPHK1)** and **sphingosine 1-phosphate (SIP)** in liver sinusoidal endothelial cell-derived exosomes has been implicated in hepatic stellate cell activation (Wang et al., 2015). Other liver sinusoidal endothelial cell-derived factors that can promote hepatic stellate cell activation includes **CXCL12** (Hong et al., 2009), while in turn hepatic stellate cell can cross-talk to liver sinusoidal endothelial cells through **VEGF**, which regulates defenestration as well as angiogenesis (Xie et al., 2012). A subset of liver sinusoidal endothelial cells may also contribute to fibrogenesis through the process of endothelial interstitial differentiation (EndMT), which is a process by which endothelial cells acquire myofibroblast phenotypes (Ribeiro et al., 2004). Dysregulated liver sinusoidal endothelial cells lose their normal immune modulatory functions, become pro-inflammatory and recruit B lymphocytes and NK cells that contribute to fibrogenesis (Feder et al., 1993; Shetty et al., 2012; Wehr et al., 2015). Finally, liver sinusoidal endothelial cells function throughout the process of liver regeneration and specifically their expression of **vascular endothelial growth factor receptor/kinase insert domain receptor (VEGFR-2)** is crucial for triggering the initial burst of hepatocyte proliferation (Ding et al., 2010). As impaired regeneration promotes fibrogenesis this vital, physiological function of liver sinusoidal endothelial cells is highly relevant.

2.2.3 | Monocyte-macrophages

Macrophages are the most abundant immune cell of the liver and are comprised from Kupffer cells and monocyte-derived macrophages (MoMϕs). Mounting evidence suggests that macrophages are heavily implicated in the pathogenesis of liver disease and are plausible druggable targets (Niu et al., 2015). Kupffer cells are located along the sinusoids and function as a primary defence against Gram-positive bacteria; in addition, they are important antigen presenting cells of the liver (Wen et al., 2021). During acute injury, Kupffer cells help to promote repair and stimulate the regenerative response. However, if repeated injury occurs, Kupffer cells stimulate the activation of hepatic stellate cells via their secretion of transforming grow factor **TGFβ1**,

PDGF and **CTGF** and promote the recruitment of inflammatory Ly6C^{high} MoMϕs, these events inducing fibrogenesis. Critical to the fibrogenic stimulation triggered by Kupffer cell activation is the elaboration of a network of C-C motif chemokine ligand (CCL) including **CCL1**, **CCL2 (MCP-1)**, **CCL5 (RANTES)** and **CCL25** (Chu et al., 2013; Karlmark et al., 2009; Miura et al., 2012; Nakamoto et al., 2012; Sasaki et al., 2017). Continuous stimulation of Ly6C^{hi} recruitment triggers a release of chemokines (CCL2, CCL5) and cytokines (**tumour necrosis factor alpha (TNFα)**, **interleukin 1-beta (IL-1β)**, **interleukin 6 |(IL-6)** and **IL-13** that promotes hepatic stellate cell trans-differentiation to their activated pro-fibrogenic phenotype and their subsequent deposition of fibrotic ECM (Wen et al., 2021). The recruitment of immature monocyte derived Ly6C^{hi} macrophages is dependent on CCL2 secreted by Kupffer cells and hepatic stellate cells (Baeck et al., 2012; Tacke, 2012). In murine fibrosis, it has been showed that Ly6C^{hi} MoMϕs promote fibrogenesis as their deletion hinders the progression of the fibrogenic response. Accordingly, immature Ly6C^{hi} CD11b^{+}/F4/80^{+} macrophages and their CCL2-dependant accumulation have a pivotal role in the initiation and progression of the fibrosis evolution. Where injury effectively resolves, MoMϕs can switch phenotype to a pro-resolution or 'restorative' Ly6C^{low} state, which can be induced either by phagocytosis or by **IL-4** and **IL-33** in the disease microenvironment (Blériot et al., 2015). Ly6C^{low} do not stimulate or maintain the myofibroblast phenotype and instead are reverted to the quiescent hepatic stellate cell phenotype or are removed by apoptosis. As a consequence, ECM deposition is halted and fibrosis can be resolved (Trautwein et al., 2015). Hence, the phenotypic switching of MoMϕs and the phenotypic features of their restorative state are receiving considerable attention as a therapeutic approach. In particular, restorative Ly6C^{low} MoMϕs express ECM degrading MMPs (**MMP9**, **12** and **13**), regenerative growth factors such as hepatocyte grow factor and insulin-like growth factor (IGF) and genes associated with phagocytes (Ramachandran et al., 2012). Moreover, an interesting autologous macrophage therapy approach has recently been suggested from the work of Stuart Forbes' laboratory involving the purification of circulating CD14^{+} monocytes from cirrhosis patients and their *ex vivo* differentiate into pro-resolution macrophages which are then infused back into the patient. A Phase 1 clinical study recently reported this to be a safe procedure and with indications of potential anti-fibrotic effects (Moroni et al., 2019). On the other hand, there has been considerable enthusiasm for the approach of blocking CCL2-mediated hepatic recruitment of Ly6C^{high} MoMϕs using **cenicriviroc**, which is a dual **CCR2**/**CCR5** antagonist (Friedman et al., 2018). However, a phase 3 trial of cenicriviroc in non-alcoholic steatohepatitis was recently terminated due to early results indicating lack of efficacy. The identification of a triggering receptor expressed on myeloid cells 2 (TREM2^{+}) CD9^{+} subpopulation of scar-associated macrophages in human cirrhotic liver by single cell RNAseq offers a new avenue of investigation, as these cells appear to stimulate collagen production by hepatic stellate cell (Ramachandran et al., 2019). In the non-cirrhotic mouse liver, TREM2 is expressed by non-parenchymal liver cells including Kupffer cells and functions to limit inflammation, immune-mediated liver damage, promote replenishment of Kupffer cells and the emergence of pro-regenerative endothelial cells following damage and protect against tumour development (Coelho et al., 2021; Esparza-Baquer et al., 2021; Perugorria et al., 2019). *TREM2* mRNA expression is elevated in the livers of patients diagnosed with non-alcoholic steatohepatitis and correlates with severity of steatosis, inflammation, hepatocyte ballooning and fibrosis (Xiong et al., 2019). In this latter report, studies in mice identified a cluster of Trem2^{hi}, Gpnmb^{+}CC9^{+} macrophages that undergo expansion during diet-induced non-alcoholic steatohepatitis and that are associated with fibrosis. In a more recent study, Hou et al. discovered that TREM2 deficient macrophages release exosomes that impair hepatocyte mitochondrial structure and function, while targeted expression of human TREM2 in mouse liver macrophages improved hepatic energy supply and prevented liver dysfunction and sepsis in a model of non-alcoholic fatty liver disease-associated sepsis (Hou et al., 2021). Hence, macrophage functions regulated by TREM2 emerge as important for non-alcoholic steatohepatitis pathogenesis and may lead to new therapeutic opportunities.

2.2.4 | T lymphocytes in fibrosis

The liver has an enriched population of NK cells, CD4^{+} and CD8^{+} T cells and γδ T cells (Jenne & Kubes, 2013). Fibrosis is suppressed by Th1 CD4^{+} T cells and promoted by Th2 cells (Wynn, 2008). IL-13 released from Th2 cells promotes hepatic stellate cell activation through the activation of TGFβ1, whereas Th1 cells at least in part suppress TGFβ1 signalling via their release of **interferon gamma (IFN-γ)** (Weng et al., 2007). The separate lineage of T helper lymphocytes known as Th17 cells is involved in the hepatic recruitment of myeloid cells via their secretion of IL17 which is also reported to activate hepatic stellate cell via mitogen activated protein kinase (MAPK) signalling pathways (Meng et al., 2012; Tan et al., 2013). CD8+ T cells in the context of obesity and non-alcoholic steatohepatitis stimulate hepatic stellate cell activation and fibrosis possibly via their production of TNFα and IL-6; these effects are not observed under non-obese conditions (Breuer et al., 2020). Activated regulatory T cells (Tregs) inhibit hepatic stellate cell activation, Th17 function and fibrosis as do γδ T cells which may mediate Fas/FasL induced hepatic stellate cell apoptosis (Li et al., 2021).

2.2.5 | Stellate cells and myofibroblast functions

The origins of fibrogenic myofibroblasts in liver fibrosis have been contentious concerning the relative contributions of epithelial to mesenchymal transition, endothelial to mesenchymal transition, infiltrating bone marrow-derived fibrocytes, activation of resident fibroblasts and hepatic stellate cell transdifferentiation. These controversies have largely been resolved through lineage tracing studies in mouse models that indicate hepatic stellate cell contributes between 82 and 90% of myofibroblasts (Iwaisako et al., 2014; Mederacke et al., 2013). Single cell RNAseq revealed the existence of zonally distributed hepatic stellate cell subpopulations, such that portal vein-associated hepatic stellate cells and central vein-associated hepatic stellate cells are now

designated as functionally distinct, with the latter being the dominant source of fibrogenic myofibroblasts (Dobie et al., 2019). Non-hepatic stellate cell-derived myofibroblasts are implicated in cholestatic fibrosis, although their origins remain controversial (Karin et al., 2016). In human cirrhotic liver, so-called scar-associated mesenchymal cells were confirmed to have hepatic stellate cell and periportal origins (Ramachandran et al., 2019). Hepatic stellate cell transdifferentiation is therefore a common event in liver disease, and there has been intense investigation concerning their fate and function (Tsuchida & Friedman, 2017). In the un-injured liver, hepatic stellate cells exist in a quiescent hepatic stellate cells state and are located in the space of Disse. The numbers of activated hepatic stellate cells increase with liver injury and by contrast, declines with resolution of injury. Activation of hepatic stellate cells is a highly regulated process, as is the removal of activated hepatic stellate cell these cells, which can occur by apoptosis and potentially be NK-cell mediated clearance of senescenthepatic stellate cells (Trivedi et al., 2020; Zhang et al., 2021). However, it is also evidenced that active hepatic stellate cells have the ability to revert either partially or completely back to an inactive hepatic stellate cells or quiescent state, and again, these are tightly regulated processes (Trivedi et al., 2020). The inherent plasticity of hepatic stellate cells holds considerably promise for the development of hepatic stellate cells-targeted treatments of fibrosis (Figure 2).

The sequence of molecular events leading to hepatic stellate cells activation has been designated into *initiation* and *perpetuation* phases that are driven by a combination of paracrine and autocrine signalling networks. Where liver injury is self-limiting or can be effectively prevented (e.g. elimination or control of hepatic stellate cell infection), then a third phase referred to as resolution also involves changes to hepatic stellate cell biology and behaviour that are pivotal for enabling the regression of fibrosis and regeneration.

2.2.6 | Initiation of hepatic stellate cells activation and fibrogenesis

The initial changes in hepatic stellate cells are a reflection of the paracrine stimulation by all neighbouring cells including Kupffer cell, MoMϕs, hepatocytes, lymphocytes, leukocytes, platelets and the sinusoidal endothelium (Kmiec, 2001). In particular, Kupffer cells play a key role through their secretion of ROS and TGFβ1, the latter being the major stimulator of hepatic stellate cell activation and matrix synthesis (Kisseleva & Brenner, 2007). Initiation is dependent on the loss of cytoplasmic lipid droplets that are characteristic of quiescent hepatic stellate cell, this involving the release of **retinol** and autophagy (Trivedi et al., 2020). Initiation also requires down-regulation of **peroxisome proliferator activated receptor gamma (PPARγ; NR1C3)**, which is controlled by the epigenetic regulators methyl-CpG binding protein 2 (MeCP2) and **enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2)** that in combination bring about repression of PPARγ gene *(PPARG)* transcription. In concert with this epigenetic repression, there is extensive remodelling of the hepatic stellate cell DNA methylome and induction of the **ASH1 like histone methyltransferase**, which is required for expression of collagen I, TIMP1 and TGFβ1 (Page et al., 2016; Perugorria et al., 2012). Alongside these epigenetic changes, initiation requires the activation of a vast number of transcription factors (Mann & Mann, 2009). Krupple-like transcription factor (KLF) family proteins Sp1, BTRB1 and KLF6 modulate the transcription of the alpha-1 collagen gene (*COL1A1*). **cAMP responsive element binding protein 1 (CREB1)** enables activated hepatic stellate cell proliferation while the transcription factors AP-1 and RUNX control the induction of TIMP-1, while Ets-1 is a downstream mediator of TGFβ-1 signalling required for induction of the fibrogenic factor **connective tissue growth factor (CTGF/CNN)**. The level of NF-κB activity is increased in activated hepatic stellate cell and not only enhances their expression of pro-inflammatory cytokines and chemokines but also promotes activated hepatic stellate cell survival (Elsharkawy et al., 2005; Lang et al., 2000). Initiation is predominantly a paracrine controlled phase of fibrogenesis in which the quiescent hepatic stellate cell is stimulated by hepatocyte-derived DAMPs including mitochondrial-derived molecules, apoptotic fragments and via the actions of TGFβ1 and PDGF that are released from infiltrating platelets and resident Kupffer cells (Bachem et al., 1992; Bilzer et al., 2006; Canbay et al., 2003) (Figure 2). The YAP1 transcriptional co-activator of the Hippo pathway is a critical driver of hepatic stellate cell activation as demonstrated by the anti-fibrotic effects of pharmacological inhibition of the pathway (Martin et al., 2016). During this early stage of hepatic stellate cell activation, the cell begins to express receptors for PDGF which is initially provided in a paracrine manner from platelets and Kupffer cell. PDGF is a highly potent mitogen for hepatic stellate cell and serves to stimulate their proliferation (Ying et al., 2017). Once the activated hepatic stellate cell is fully matured, it produces its own PDGF along with other mitogens, which then marks transition to the perpetuation phase.

2.2.7 | Perpetuation

The paracrine stimuli described above continue to be supplied during perpetuation of hepatic stellate cell activation. However, once the hepatic stellate cell adopts its mature α α-smooth muscle actin+ myofibroblast-like phenotype, it has the capability to self-promote many of its key fibrogenic characteristics such as proliferation, survival, chemotactic migration, contractility, ECM deposition, maturation and turnover and recruitment of leukocytes. Autocrine cytokines such as $TGF\beta_1$, PDGF, **fibroblast growth factors (FGFs)**, CTGF, **endothelin-1 (ET-1)** and cytokines inhibiting hepatic stellate cells activation such as **hepatocyte growth factor (HGF)** start to be expressed by the activated hepatic stellate cell. In this phase, activated hepatic stellate cells also release neutrophil and monocyte chemo-attractants including colony stimulating factor, monocyte chemotactic protein-1 (MCP-1) (Marra et al., 1993, 1998) and cytokine-induced neutrophil chemoattractant/IL8 (Maher et al., 1998), which promote inflammation. Interestingly, activated hepatic stellate cells also secrete anti-inflammatory cytokines, such as **IL-10**. ECM remodelling continues during hepatic stellate cell activation as during this phase, low-density

subendothelial matrix is progressively replaced by one that is rich in fibril-forming collagens. This fundamental shift in ECM composition affects the phenotype of the hepatocytes, sinusoidal endothelium and hepatic stellate cells (Figure 2). Fibril forming ECM also stimulates hepatic stellate cell activation, this mediated by the biding of fibrillar collagen to the surface of hepatic stellate cell via the **discoidin domain receptor tyrosine kinase 2 (DDR2)** and **integrins**. DDR2 has been identified as an up-regulated TK receptor. This has the characteristic property of responding to fibrillar collagen (Olaso, 1999; Olaso et al., 2001) by activating **SRC proto-oncogene, non-receptor tyrosine kinase (Src)** and downstream signals that culminate in transcriptional induction of **MMP2** (Ikeda et al., 2002). Recent studies in our laboratory have identified a previously unrealised role for TGFB1 as a stimulator of a pro-fibrogenic phenotype for hepatocytes (Leslie et al., 2020). TGFB1 exposed hepatocytes undergo activation of the NF-κB transcription factor c-Rel which stimulates glycolysis via increased expression of the glycolytic enzyme **6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3)** his increased glycolytic activity is required for the 'activated' hepatocyte to secrete soluble profibrogenic molecules including CTGF, **cathepsin D**, **bone morphogenetic protein (BMP1)** and serpin 1. Genetic and pharmacological targeting of c-Rel or PFKFB3 was shown to be anti-fibrotic and pro-regenerative (Leslie et al., 2020).

2.2.8 | Resolution

Multiple mechanisms contribute to the removal of collagen-producing activated hepatic stellate cell during the resolution phase. The concept of reversion to a quiescent phenotype has been described in the literature for many years and initially focussed on reconstitution of PPARγ signalling as a means to halt or reverse hepatic stellate cell activation (Miyahara et al., 2000; Yang et al., 2006). Recent studies using *in vivo* hepatic stellate cell-specific ablation of PPARγ confirmed an important role for the nuclear receptor in fibrosis regression with the observation that upon cessation of liver injury, fibrosis regression was slower and associated with maintenance of elevated hepatic collagen and α-smooth muscle actin expression (Liu et al., 2020). Moreover, in this same study, administration of the PPARγ agonist **rosiglitazone** accelerated fibrosis regression. Nakano et al. (2020) recently described how experimental over-expression of the transcription factor TCF21 in activated hepatic stellate cell suppressed fibrogenic gene expression and partially restored their quiescent phenotype both *in vitro* and *in vivo*. Cell–cell interactions have also been implicated in the control of reversion of activated hepatic stellate cell phenotype; in particular, restoration of liver sinusoidal endothelial cell differentiation promotes hepatic stellate cell quiescence and regression of fibrosis (Xie et al., 2012). As quiescent hepatic stellate cell in turn promotes liver sinusoidal endothelial cell differentiation (DeLeve et al., 2004) further focus on the biology of hepatic stellate cell-liver sinusoidal endothelial cell cross-talk should illuminate novel strategies to promote fibrosis regression and restoration of normal sinusoidal structure and function.

In addition to activated hepatic stellate cell having the capacity to revert to quiescence, lineage tracing studies in mouse models of fibrosis reversion discovered that they can also undergo epigenetic and transcriptional reprogramming into a distinct so-called 'inactive' hepatic stellate cell or deactivated phenotype (Kisseleva et al., 2012; Troeger et al., 2012). These inactive hepatic stellate cell may persist for up to 45 days following fibrosis regression and are susceptible to reactivation upon a subsequent round of liver injury, this suggesting that inactive hepatic stellate cell may provide a reservoir of primed or pre-activated hepatic stellate cell. Liu and colleagues (2020) have confirmed the human relevance of hepatic stellate cell inactivation by demonstrating with elegant *in vivo* studies that human activated hepatic stellate cell transplanted into mouse liver can be recovered with an inactive hepatic stellate cell phenotype that was associated with elevated expression of PPARγ and decreased expression of ***Col1α1*** and α-smooth muscle actin transcripts. While approximately 50% of activated hepatic stellate cell undergo deactivation or reversion to quiescence, the remaining activated hepatic stellate cells are cleared by apoptosis and/or NK-mediated killing of senescent cells (Nakano et al., 2020; Trivedi et al., 2020). The activated hepatic stellate cell up-regulates a variety of autocrine pathways that prevents apoptosis including activation of an **angiotensin II**/NF-κB positive feedback pathway TIMP-1, TGFβ and pro-inflammatory stimulators of NF-κB. Pharmacological inhibition of NF-κB brings about activated hepatic stellate cell apoptosis and accelerates fibrosis regression (Anan et al., 2006; Elsharkawy et al., 2005; Oakley et al., 2005; Wright et al., 2001). Taken together, the discovery of activated hepatic stellate cell deactivation, reversion to quiescence and susceptibility to pharmacological-induced apoptosis suggests that experimental manipulation of hepatic stellate cell plasticity holds considerable promise for future therapeutic development.

2.3 | Fibrolysis and regeneration

The experimental and clinical evidence for regression of fibrosis is compelling, and indeed, there is striking observational data to suggest that even human cirrhosis may to a certain extent be reversible (Marcellin et al., 2013). Remarkably, collagens make up to 50% of the dry weight of cirrhotic liver; however, changes in the hepatic ECM with fibrosis are more complex than simple collagen accumulation involving qualitative changes in the composition of distinct collagen molecules as well as non-collagen ECM proteins (e.g. increases in elastin, fibulin, microfibril-associated glycoprotein 4 (MFAP-4), **vitronectin**, **fibronectin**, etc.). These changes alter the stiffness of the liver, which can further drive synthesis and deposition of fibrotic ECM components due to stimulatory effects on TGFβ signalling and hepatic stellate cell fibrogenic behaviour (Karin et al., 2016; Kisseleva & Brenner, 2008). It is therefore evident that breakdown of fibrotic ECM (fibrolysis) has the potential to halt fibrogenesis and stimulate fibrosis reversion. Fibrosis reversion has been observed where there has been an effective intervention for the underlying liver injury, this being observed in the context of hepatitis B virus, hepatitis C virus,

autoimmune hepatitis, hemochromatosis, alcoholic hepatitis, bile duct obstruction, primary biliary cholangitis and non-alcoholic steatohepatitis (Ellis & Mann, 2012; Rockey, 2019). Resolution of inflammation precedes regression of fibrosis and tissue regeneration involving key functions for regulatory T cells, which facilitate termination of inflammation and restorative macrophages which express MMPs required for ECM remodelling (Pellicoro et al., 2014). Removal of activated hepatic stellate cell by apoptosis, inactivation or reversion to quiescence leads to diminution of hepatic TIMP1 and loss of its broad inhibitory activity against ECM-degrading MMPs. However, the precise nature of the fibrolytic process that drives reversion of fibrosis remains relatively unexplored. Fibrogenesis is antagonistic of regeneration and vice versa (Suarez-Cuenca et al., 2008), but again the biology underlying this relationship remains to be fully defined. **5-hydroxytryptamine (5-HT; serotonin)** can have opposing actions, either stimulating regeneration by direct activation of **5-HT$_{2B}$ receptors** on hepatocytes or by repressing regeneration and promoting fibrogenesis through activation of TGFβ1 production by hepatic stellate cell (Ebrahimkhani et al., 2011).

Regression of fibrosis alongside termination of inflammation enables regeneration of hepatocytes and cholangiocytes. However, the capacity for this regenerative process can be severely limited in chronic liver disease due to replicative senescence of hepatic epithelial cells (Bird et al., 2018; Ferreira-Gonzalez et al., 2018). Under these conditions, so-called *bipotential epithelial cells* of biliary origin can be differentiated into hepatocytes and indeed there is also evidence for hepatocyte-to-cholangiocyte differentiation under the control of Sox9, which can be up-regulated in hepatocytes located in periportal regions (Campana et al., 2021). Liver regeneration additionally requires restoration of the normal hepatic vasculature and sinusoidal structure as demonstrated by the observation that **fms related receptor tyrosine kinase 1 (VGFR-1; gene Fit1)** knockout mice exhibit delays in regeneration (Kato et al., 2011). However, maladaptive neovascularisation in the context of unresolved liver injury is a typical pathological feature of the cirrhotic liver underlying the development of hyperdynamic circulatory syndrome and portal hypertension (Garbuzenko & Arefyev, 2017). Major vascular changes in the cirrhotic liver are an impediment to restoration of normal liver structure and function even where damage, inflammation and fibrosis are effectively resolved, this establishing a 'point of no return' in cirrhosis.

3 | THERAPEUTIC STRATEGIES IN LIVER FIBROSIS

3.1 | Anti-fibrotic therapeutic challenges in chronic liver disease

There is a wealth of clinical evidence that liver fibrosis can be halted and that regression of fibrotic ECM is achievable and enables restoration of normal liver architecture and function (Ellis & Mann, 2012). However, this evidence is provided from clinical studies and real-world cases in which effective and sustained resolution of the primary cause of liver damage is achieved rather than from any direct pharmacological manipulation of the fibrotic process per se. Hence, we are lacking strong clinical evidence that liver fibrosis is a pharmacologically manipulable pathology, indeed at the present time there are only two clinically approved anti-fibrotic medicines, these being **pirfenidone** and **nintedanib** that are licensed for the treatment of idiopathic pulmonary fibrosis, the latter being a very aggressive respiratory pathology for which gaining a few additional months of life for the patient is considered a desirable treatment outcome (Cameli et al., 2020). Clearly, given that the majority of chronic liver disease patients can expect to live for at least several years and in many cases for decades, the challenge of developing safe and efficacious anti-fibrotic medicines is considerable.

At the present time, there are no clinically approved medicines that are proven effective for either preventing, halting or reversing liver fibrosis in patients. This despite a wealth of knowledge available regarding the pathobiology of liver fibrosis, a plethora of published examples of drugs with anti-fibrotic activities in experimental models of liver disease and considerable recent clinical trial activity using new drug candidates (Noureddin & Sanyal, 2018; Vuppalanchi et al., 2021). Major hurdles to overcome towards improvement of clinical trial design include the problem of disease heterogeneity, particularly in terms of the rate of progression of fibrosis and potential for fibrosis regression both of which may dramatically differ between individual patients. The relatively short time period over which clinical trials are carried out when considering that fibrosis can in many patients be slow to progress and regress. The lack of minimal invasive biomarkers for the selection of patients who are most likely to benefit from anti-fibrotics and for monitoring disease progress during a clinical trial; lack of affordable fibrosis imaging modalities that provide an organ-wide assessment of fibrosis as an adjunct or alternative to a liver biopsy; and finally, intra- and inter-observer errors that are inherent in pathology grading of liver biopsies. The major drug design and development hurdles include target validation at the pre-clinical stage with a particular emphasis on selecting pre-clinical models that provide a robust and pathophysiologically close imitation of human liver disease mechanisms; identification and validation of drug targets that will selectively engage with a critical fibrogenic pathway within the fibrotic microenvironment; avoiding toxicity in patients who may have to be treated for decades and in the context of ongoing liver injury which can impact on drug metabolism and mitigating against the potential of a drug to impair liver regeneration and/or promote cancer. It is also highly likely that the background cause of liver damage and its impact on liver physiology, metabolism and immunology will need to be considered as and when new anti-fibrotics enter the clinic.

The ideal anti-fibrotic for treatment of chronic liver disease would be evidenced as selectively engaging with its target molecular pathway, clinically achieving at least a 1-grade regression of fibrosis in patients diagnosed with advanced bridging fibrosis and in the absence of adverse events that would preclude the patient withdrawing from treatment for at least 2 or 3 years. In addition, the drug would need to

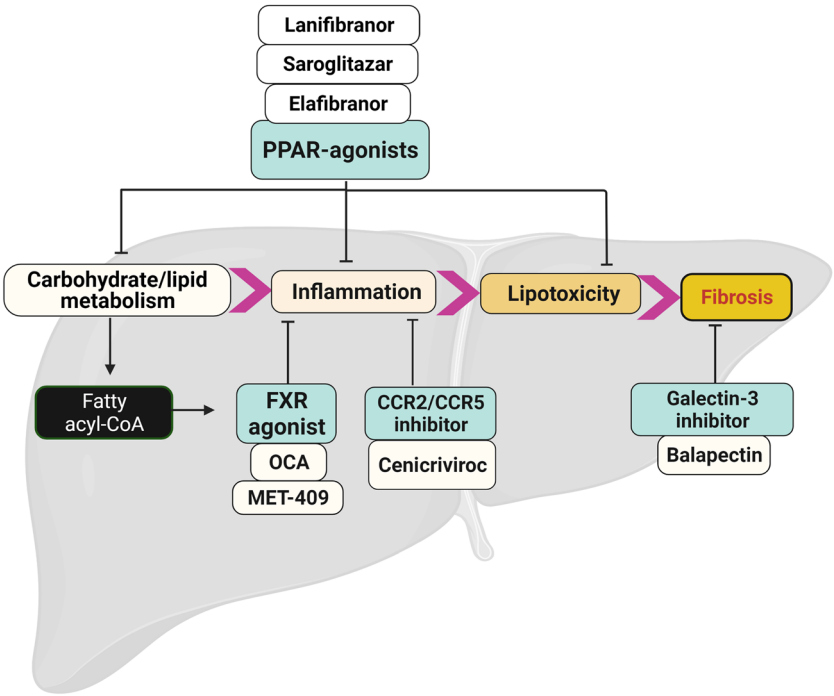

FIGURE 3 Scheme of drugs that are in phase II and phase III development and their targets. Agonists of PPRs and FXR, inhibitors of CCR2/CCR5 and Gal3 are the most promising agents for fibrosis therapeutic interventions

avoid causing a liver injury as assessed by pathology on a post-treatment biopsy sample and to be tolerable in the context of any other medication that patients may be taking for their underlying disease or co-morbidities that are common in chronic liver disease and especially where liver function and metabolism are compromised. The challenge to the pharmaceutical industry is therefore considerable, however given the high and rising global incidence of chronic liver disease and the urgent unmet need for a safe and efficacious anti-fibrotic the rewards of meeting the challenge are sufficiently high for this to remain a highly active area of R&D for the industry over the next decade and more.

3.2 | The current state-of-play for anti-fibrotic therapeutics

The vast majority of clinical trials in liver fibrosis over the past 5 year have focused on non-alcoholic steatohepatitis in which the presence of fibrosis on the background on steatohepatitis is considered a high risk for progression to cirrhosis (Singh et al., 2015) (Figure 3). Patients are selected for trials on the basis of evidence of advanced fibrosis in a liver biopsy and undergo a repeat biopsy together with other clinical and biomarker measurements following a period of a year or more of treatment with an experimental drug. A confounder for the success of clinical trial studies in non-alcoholic steatohepatitis is a high rate of placebo effect with up to 22% of patients undergoing an improvement in fibrosis score, which may be due to a variety of factors including biopsy sampling and life style modifications (Noureddin & Sanyal, 2018). A further contributory factor to the high rate of attrition of promising new treatments for non-alcoholic steatohepatitis is the lack of pre-clinical models that can accurately recapitulate human non-alcoholic steatohepatitis. Despite these limitations there continues to be increased clinical trial activity in non-alcoholic steatohepatitis with many ongoing phase 2 and 3 trials.

3.2.1 | Drugs targeting farnesoid X receptor (FXR; NRIH4)

FXR is a master regulator of bile acid homeostasis and metabolism, including synthesis, secretion, conjugation, absorption and refilling of the gall bladder. It is highly expressed in the liver and small intestine, a characteristic that makes it a good candidate for drug development in liver pathologies. When activated by bile acids, FXRs regulate lipid and glucose homeostasis, promote insulin sensitivity and potentially, modulate liver fibrosis (Mudaliar et al., 2013; Neuschwander-Tetri et al., 2015). **Obeticholic acid**, an orally available semisynthetic steroidal FXR agonist, has been tested in non-alcoholic steatohepatitis models (humans and mouse) and pre-clinical models of cholestasis and fibrosis. The compound showed good efficiency in promoting insulin sensitivity and decreasing markers of liver inflammation and fibrosis in patients affected by type II diabetes and non-alcoholic steatohepatitis (Mudaliar et al., 2013; Neuschwander-Tetri et al., 2015). The FLINT phase II clinical study revealed that 45% of patients with non-alcoholic steatohepatitis who were randomised to obeticholic acid showed improved liver histology compared to the placebo group. Importantly, 35% of patients taking obeticholic acid (25 mg/day of obeticholic acid had improved fibrosis (Neuschwander-Tetri et al., 2015). Surprisingly, 19% of patients on the placebo arm displayed improved fibrosis by one or more grades and overall, the effect of obeticholic acid over the placebo was limited (Neuschwander-Tetri et al., 2015). Moreover, obeticholic acid promoted pruritus and it is

unclear in a 'real-world' scenario how many individuals would discontinue therapy due to itching. Another adverse effect associated with obeticholic acid intake is the increased level of LDL and very low-density lipoproteins (VDL) particles and reduction of HDL particles after 12 weeks of therapy (Younossi et al., 2019). The main medical management to control hyperlipidaemia was the addition of statin medications.

The itching consequent to obeticholic acid intake was attributed to the steroidal structure of the molecule, which is associated with agonistic effects on the G-protein coupled **bile acid receptor 1 (GBPA receptor**, also known as TGR5) (Alemi et al., 2013). This had let to the synthesis of several non-steroidal FXR agonist, which are now being evaluated (e.g. **cilofexor**, **tropifexor**, EDP-305, MET-409), in order to preserve therapeutic potential and minimise adverse effects. However, contrary to expectations, Phase II clinical trials evaluating synthetic agonists continue to find a dose response association with pruritus (Vuppalanchi et al., 2021). Among the new structurally optimised synthetic FXR agonists, MET-409 was recently clinically tested in 58 patients with non-alcoholic steatohepatitis or fibrosis having ≥10% liver fat, during 12 weeks at 50-mg or 80-mg dose. Relative mean fat was significantly reduced compared to placebo (55%, 38% and 6% less in 80 mg, 50 mg and placebo, respectively). Approximately 93% of patients in the trial showed decreased baseline liver fat and decrease in alanine aminotransferase (ALT). However, a subgroup of patients developed (at both doses), a transient increase in ALT levels. The compound was also associated with pruritus, but with reduced severity when compared to the other non-steroidal obeticholic acid (Harrison et al., 2021; Kremoser, 2021).

3.2.2 | Drugs targeting peroxisome **proliferator-activated receptors (PPARs)**

PPARs are ligand-activated transcription factors that regulate several metabolic process (Grygiel-Górniak, 2014; Macdonald & Prins, 2004). Several molecules have been conceived as agonist of the PPAR receptors. **PPARα (NR1C1)** is expressed in metabolically active tissue and once activated lowers lipid levels, drives the expression of lipogenic gene and promotes the transcription of **FGF21**, which can itself induce beneficial metabolic changes and is an interesting drug target in non-alcoholic steatohepatitis (Musso et al., 2016). PPARα agonists have been reported to decrease numbers of pro-fibrogenic macrophages and hepatic stellate cell leading to reduced hepatic expression of fibrogenic markers (Ip et al., 2003). However, the major hepatoprotective effects of PPARα agonism is suggested to be mainly through actions on liver parenchymal cells (Pawlak et al., 2015). **PPARβ/δ (NR1C2)** is highly expressed in hepatocytes and is involved in decreasing hepatic glucose production, stimulating insulin sensitivity and fatty acid oxidation. PPARβ/δ also exerts anti-inflammatory activities in macrophages via direct inhibition of NF-κB (Zingarelli et al., 2010). Elafibranor is a dual agonist for PPAR-α/PPAR-δ receptors and participates in the regulation of lipid metabolism and glucose homeostasis. Phase II studies in steatotic non-cirrhotic patients receiving 120 mg of Elafibranor for 52 weeks demonstrated a reversal of non-alcoholic steatohepatitis without worsening fibrosis (Albhaisi & Sanyal, 2021). However, interim data from the Phase III trial RESOLVE-IT indicated a failure of the drug to resolve non-alcoholic steatohepatitis (https://ir.genfit.com/news-releases/news-release-details/genfit-announces-results-interim-analysis-resolve-it-phase-3/) resulting in termination of the study. PPARγ is mechanistically more directly associated with fibrogenesis, in particular it functions as a repressor of hepatic stellate cell activation and can stimulate the inactivation of hepatic stellate cell and promote fibrosis regression (Liu et al., 2020; Ni et al., 2021). Saroglitazar is a dual agonist for PPAR-α and PPAR-γ, which has shown pre-clinical promise in non-alcoholic steatohepatitis and is now in clinical assessment (NCT03061721) (Jain et al., 2018; Kaul et al., 2019). Lanifibranor (IVA337) is a pan-PPAR agonist that has been designated fast-track status for non-alcoholic steatohepatitis with evidence from the Phase IIb NATIVE trial of achieving a decrease in the SAF (steatosis, activity and fibrosis) score. A second trial is now recruiting patients with type 2 diabetes and non-alcoholic fatty liver disease (Pierre et al., 2020).

3.2.3 | Balapectin—an inhibitor of **galectin 3 (Gal-3)**

Gal-3 is a lectin that has both intracellular and extracellular functions, and is of particular interest in fibrosis because of its contributions to cell-matrix interactions, cell growth and macrophage activation (Hara et al., 2020). Gal-3 is expressed by Kupffer cell and is inducibly expressed during hepatic stellate cell activation. Mice lacking Gal-3 are protected from liver fibrosis and display defects in hepatic stellate cell activation (Pugliese et al., 2015; Traber & Zomer, 2013). Increased hepatic expression of Gal-3 is observed in human non-alcoholic steatohepatitis, which led to the use of a complex carbohydrate inhibitor Balapectin in a Phase IIb trial in 162 patients with non-alcoholic steatohepatitis-cirrhosis that included portal hypertension, which is a major risk factor for mortality. Although balapectin did not achieve a significant reduction for fibrosis, a subgroup of patients with oesophageal varices did show an improvement in hepatic venous pressure gradient (Chalasani et al., 2020). On that basis and the low toxicity of balapectin, a Phase III trial is to be initiated.

3.2.4 | Chemokine receptor antagonism

The CCR2 and CCR5 chemokine receptors are implicated in the recruitment of pro-fibrogenic monocytes to the damaged liver which promotes and perpetuates hepatic stellate cell activation (Tacke, 2012). Cenicriviroc, a dual antagonist for CCR2 and CCR5, showed considerable promise at Phase IIb with good tolerability and improvement in fibrosis in non-alcoholic steatohepatitis patients (Ratziu et al., 2020). However, following lack of efficacy for improvement of fibrosis in part 1 of the AURORA (NCT03028740) Phase III trial, the future use of this therapeutic strategy in non-alcoholic steatohepatitis is in question.

3.2.5 | Future direction

The current standard of treatment of chronic liver disease caused by steatosis (alcohol or dietary) is lifestyle modification. This mostly involves abstinence from consumption of alcohol or caloric reduction, and increase in physical activity to sustain weight loss, with the aim of reducing liver inflammation and slowing down, halting or reversing fibrosis progression. Although several existing medications have the potential to play a role in fibrosis therapy, none is currently FDA approved medications. An increasing number of promising drug candidates are failing to meet the primary end-points of trials when administered as monotherapy and many pharmaceutical companies are looking at the potential for combination therapies with 2 or more compounds targeting different pathways simultaneously. However, these combination approaches will need to carefully consider potential for adverse events resulting from modification of more than one biological mechanism.

Basic research in fibrosis is continuously discovering new routes involved in the development of fibrosis. As an example, epigenetic drugs may hold the potential of becoming possible new therapeutic agents. DNA methylation, histone post translational modification and non-coding RNAs have altered expression and function in the context of liver fibrosis and there are a plethora of small molecule inhibitors of these mechanisms emerging as candidates for therapeutic modulation of fibrosis (Barcena-Varela et al., 2021; Wilson et al., 2017; Zeybel et al., 2017). As many epigenetic factors that are implicated in liver fibrosis play essential functions in a variety of cell types, it is likely that targeting of epigenetic drugs will be required, as an example, our group have previously reported the use of immune-nanoparticles to direct inhibition of histone methylation in hepatic stellate cells which delivered a therapeutic effect in the context of a murine model of liver damage (Zeybel et al., 2017). The inflammasome is another area of considerable interest which with recent improved understanding of macrophage subsets involved in fibrosis activation and resolution may pave the way for new approaches targeting fibrolysis (Vuppalanchi et al., 2021). Signalling pathways activated by endoplasmic reticulum stress that are collectively termed as the unfolded protein response (UPR) may also provide novel therapeutic targets. The UPR is directed via three distinct pathways regulated by the signalling molecules **endoplasmic reticulum to nucleus signaling 1 (IRE1)**, **eukaryotic translation initiation factor 2 alpha kinase 3 (PERK)** and activating transcription factor 6 alpha (ATF6α; Maiers & Malhi, 2019). All three of these UPR pathways are activated in non-alcoholic fatty liver disease and moreover the UPR is found to be active in hepatic stellate cells following liver injury. This is most likely to be due to their response to ROS and their requirement for increased secretion of ECM constituents. Endoplasmic reticulum stress is also intrinsically related with the inflammatory response and the crosstalk between in UPR and the inflammasome remains debated at this time. (Zhang et al., 2016). Precisely how the UPR can be targeted in liver fibrosis and in which cell types remains to be determined and would most likely require targeted approaches. An additional promising area of research that is worthy of further consideration is the therapeutic potential for extracellular vesicles and in particular exosomes that are derived from mesenchymal stem cells (MSC) (Lee et al., 2021). MSCs may promote resolution of liver fibrosis via multiple mechanisms including replacement of lost hepatocytes with differentiated hepatocyte-like cells, stimulating hepatic stellate cell apoptosis and anti-inflammatory activities (Berardis et al., 2015; Eom et al., 2015; Lee et al., 2021). However, MSC therapy has limitations for human applications including poor engraftment following transplantation and cancer risks (Quante et al., 2011; Von Bahr et al., 2012). In the carbon tetrachloride model of liver fibrosis, MSC-derived exosomes are reported to suppress profibrogenic TGFβ and **Wnt/β-catenin** signalling, decrease oxidative stress and stimulate hepatocyte proliferation (Jiang et al., 2018; Li et al., 2013; Rong et al., 2019). However, there are considerable obstacles preventing the clinical application of exosomes and indeed other types of extracellular vesicles including improving their stability and tissue targeting.

4 | SUMMARY

Despite decades of research and an increasing understanding of the complexities in the cell and molecular biology of liver fibrosis, the pathology remains a difficult clinical challenge that is in urgent need of safe and efficacious therapeutic approaches that halt or reverse its progression to end stage liver disease or cancer. This aim will require the application of advanced single cell genomics, epigenomics and proteomics technologies that provide greater resolution of novel therapeutic targets, the design and application of pre-clinical models of chronic liver disease that better recapitulate human disease, such as precision cut human liver slices (Paish et al., 2019) and the development of patient stratification tools that enable improved clinical trial design, in particular with regard to the select of those patients in which fibrosis is rapidly progressing but can be responsive to targeted intervention.

4.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander, Christopoulos, et al., 2021; Alexander, Fabbro, Kelly, Mathie, Peters, Veale, Armstrong, Faccenda, Harding, Pawson, Southan, Davies, Beuve, et al., 2021; Alexander, Fabbro, Kelly, Mathie, Peters, Veale, Armstrong, Faccenda, Harding, Pawson, Southan, Davies, Boison, et al., 2021).

CONFLICT OF INTEREST

Prof. Derek Mann is a Director, employee and major shareholder of FibroFind Ltd.

ORCID

Maria Teresa Borrello https://orcid.org/0000-0003-1365-1358

REFERENCES

Adams, L. A. (2011). Biomarkers of liver fibrosis. *Journal of Gastroenterology and Hepatology*, *26*, 802–809.

Albhaisi, S. A. M., & Sanyal, A. J. (2021). New drugs for NASH. *Liver International*, *41*, 112–118.

Alemi, F., Kwon, E., Poole, D. P., Lieu, T., Lyo, V., Cattaruzza, F., Cevikbas, F., Steinhoff, M., Nassini, R., Materazzi, S., Guerrero-Alba, R., Valdez-Morales, E., Cottrell, G. S., Schoonjans, K., Geppetti, P., Vanner, S. J., Bunnett, N. W., & Corvera, C. U. (2013). The TGR5 receptor mediates bile acid–induced itch and analgesia. *The Journal of Clinical Investigation*, *123*, 1513–1530.

Alexander, S. P., Christopoulos, A., Davenport, A. P., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Abbracchio, M. P., Alexander, W., Al-hosaini, K., Bäck, M., Barnes, N. M., Bathgate, R., … Ye, R. D. (2021). THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: G protein-coupled receptors. *British Journal of Pharmacology*, *178*(S1), S27–S156. https://doi.org/10.1111/bph.15538

Alexander, S. P., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Beuve, A., Brouckaert, P., Bryant, C., Burnett, J. C., Farndale, R. W., Friebe, A., Garthwaite, J., … Waldman, S. A. (2021). THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: Catalytic receptors. *British Journal of Pharmacology*, *178*(S1), S264–S312. https://doi.org/10.1111/bph.15541

Alexander, S. P., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Boison, D., Burns, K. E., Dessauer, C., Gertsch, J., Helsby, N. A., Izzo, A. A., Koesling, D., … Wong, S. S. (2021). THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: Enzymes. *British Journal of Pharmacology*, *178*(S1), S313–S411. https://doi.org/10.1111/bph.15542

Anan, A., Baskin-Bey, E. S., Bronk, S. F., Werneburg, N. W., Shah, V. H., & Gores, G. J. (2006). Proteasome inhibition induces hepatic stellate cell apoptosis. *Hepatology*, *43*, 335–344.

Arriazu, E., Ruiz de Galarreta, M., Cubero, F. J., Varela-Rey, M., Pérez de Obanos, M. P., Leung, T. M., Lopategi, A., Benedicto, A., Abraham-Enachescu, I., & Nieto, N. (2014). Extracellular matrix and liver disease. *Antioxidants & Redox Signaling*, *21*, 1078–1097.

Arshad, T., Golabi, P., Henry, L., & Younossi, Z. M. (2020). Epidemiology of non-alcoholic fatty liver disease in North America. *Current Pharmaceutical Design*, *26*, 993–997.

Bachem, M. G., Meyer, D., Melchior, R., Sell, K.-M., & Gressner, A. M. (1992). Activation of rat liver perisinusoidal lipocytes by transforming growth factors derived from myofibroblastlike cells. A potential mechanism of self perpetuation in liver fibrogenesis. *The Journal of Clinical Investigation*, *89*, 19–27.

Baeck, C., Wehr, A., Karlmark, K. R., Heymann, F., Vucur, M., Gassler, N., Huss, S., Klussmann, S., Eulberg, D., Luedde, T., Trautwein, C., & Tacke, F. (2012). Pharmacological inhibition of the chemokine CCL2 (MCP-1) diminishes liver macrophage infiltration and steatohepatitis in chronic hepatic injury. *Gut*, *61*, 416–426.

Banales, J. M., Huebert, R. C., Karlsen, T., Strazzabosco, M., LaRusso, N. F., & Gores, G. J. (2019). Cholangiocyte pathobiology. *Nature Reviews Gastroenterología Y Hepatología*, *16*, 269–281.

Barcena-Varela, M., Paish, H., Alvarez, L., Uriarte, I., Latasa, M. U., Santamaria, E., Herranz, J. M., Santos-Laso, A., Arechederra, M., de Gauna, M. R., Aspichueta, P., Canale, M., Casadei-Gardini, A., Francesconi, M., Carotti, S., Morini, S., Nelson, L. J., Iraburu, M. J., Chen, C., … Avila, M. A. (2021). Epigenetic mechanisms and metabolic reprogramming in fibrogenesis: Dual targeting of G9a and DNMT1 for the inhibition of liver fibrosis. *Gut*, *70*, 388–400.

Basisty, N., Kale, A., Jeon, O. H., Kuehnemann, C., Payne, T., Rao, C., Holtz, A., Shah, S., Sharma, V., Ferrucci, L., Campisi, J., & Schilling, B. (2020). A proteomic atlas of senescence-associated secretomes for aging biomarker development. *PLoS Biology*, *18*, e3000599.

Bataller, R., & Brenner, D. A. (2005). Liver fibrosis. *The Journal of Clinical Investigation*, *115*, 209–218. https://www.jci.org/articles/view/24282

Berardis, S., Sattwika, P. D., Najimi, M., & Sokal, E. M. (2015). Use of mesenchymal stem cells to treat liver fibrosis: Current situation and future prospects. *World Journal of Gastroenterology: WJG*, *21*, 742.

Bilzer, M., Roggel, F., & Gerbes, A. L. (2006). Role of Kupffer cells in host defense and liver disease. *Liver International*, *26*, 1175–1186.

Bird, T. G., Müller, M., Boulter, L., Vincent, D. F., Ridgway, R. A., Lopez-Guadamillas, E., Lu, W.-Y., Jamieson, T., Govaere, O., Campbell, A. D., Ferreira-Gonzalez, S., Cole, A. M., Hay, T., Simpson, K. J., Clark, W., Hedley, A., Clarke, M., Gentaz, P., Nixon, C., … Forbes, S. J. (2018). TGFβ inhibition restores a regenerative response in acute liver injury by suppressing paracrine senescence. *Science Translational Medicine*, *10*, eaan1230. https://doi.org/10.1126/scitranslmed.aan1230

Blériot, C., Dupuis, T., Jouvion, G., Eberl, G., Disson, O., & Lecuit, M. (2015). Liver-resident macrophage necroptosis orchestrates type 1 microbicidal inflammation and type-2-mediated tissue repair during bacterial infection. *Immunity*, *42*, 145–158.

Breuer, D. A., Pacheco, M. C., Washington, M. K., Montgomery, S. A., Hasty, A. H., & Kennedy, A. J. (2020). CD8+ T cells regulate liver injury in obesity-related nonalcoholic fatty liver disease. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *318*, G211–G224.

Cameli, P., Refini, R. M., Bergantini, L., d'Alessandro, M., Alonzi, V., Magnoni, C., Rottoli, P., Sestini, P., & Bargagli, E. (2020). Long-term follow-up of patients with idiopathic pulmonary fibrosis treated with pirfenidone or nintedanib: A real-life comparison study. *Frontiers in Molecular Biosciences*, *7*, 230.

Campana, L., Esser, H., Huch, M., & Forbes, S. (2021). Liver regeneration and inflammation: From fundamental science to clinical applications. *Nature Reviews. Molecular Cell Biology*, *22*, 608–624.

Canbay, A., Feldstein, A. E., Higuchi, H., Werneburg, N., Grambihler, A., Bronk, S. F., & Gores, G. J. (2003). Kupffer cell engulfment of apoptotic bodies stimulates death ligand and cytokine expression. *Hepatology*, *38*, 1188–1198.

Chalasani, N., Abdelmalek, M. F., Garcia-Tsao, G., Vuppalanchi, R., Alkhouri, N., Rinella, M., Noureddin, M., Pyko, M., Shiffman, M., Sanyal, A., Allgood, A., Shlevin, H., Horton, R., Zomer, E., Irish, W., Goodman, Z., Harrison, S. A., Traber, P. G., & Belapectin (GR-MD-02) Study Investigators. (2020). Effects of belapectin, an inhibitor of galectin-3, in patients with nonalcoholic steatohepatitis with cirrhosis and portal hypertension. *Gastroenterology*, *158*, 1334–1345.

Chen, W., Yang, A., Jia, J., Popov, Y. V., Schuppan, D., & You, H. (2020). Lysyl oxidase (LOX) family members: Rationale and their potential as therapeutic targets for liver fibrosis. *Hepatology*, *72*, 729–741.

Chowdhury, U. R., Samant, R. S., Fodstad, O., & Shevde, L. A. (2009). Emerging role of nuclear protein 1 (NUPR1) in cancer biology. *Cancer Metastasis Reviews*, *28*, 225–232.

Chu, P., Nakamoto, N., Ebinuma, H., Usui, S., Saeki, K., Matsumoto, A., Mikami, Y., Sugiyama, K., Tomita, K., Kanai, T., Saito, H., & Hibi, T. (2013). C-C motif chemokine receptor 9 positive macrophages activate hepatic stellate cells and promote liver fibrosis in mice. *Hepatology*, *58*, 337–350.

Coelho, I., Duarte, N., Barros, A., Macedo, M. P., & Penha-Gonçalves, C. (2021). Trem-2 promotes emergence of restorative macrophages and endothelial cells during recovery from hepatic tissue damage. *Frontiers in Immunology*, *11*, 3784.

Dam-Larsen, S., Franzmann, M., Andersen, I. B., Christoffersen, P., Jensen, L. B., Sørensen, T. I. A., Becker, U., & Bendtsen, F. (2004). Long term prognosis of fatty liver: Risk of chronic liver disease and death. *Gut*, *53*, 750–755.

DeLeve, L. D., Wang, X., Hu, L., McCuskey, M. K., & McCuskey, R. S. (2004). Rat liver sinusoidal endothelial cell phenotype is maintained by paracrine and autocrine regulation. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *287*, G757–G763.

Ding, B.-S., Nolan, D. J., Butler, J. M., James, D., Babazadeh, A. O., Rosenwaks, Z., Mittal, V., Kobayashi, H., Shido, K., Lyden, D., Sato, T. N., Rabbany, S. Y., & Rafii, S. (2010). Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration. *Nature*, *468*, 310–315.

Ding, J., & Wang, H. (2014). Multiple interactive factors in hepatocarcinogenesis. *Cancer Letters*, *346*, 17–23.

Dobie, R., Wilson-Kanamori, J. R., Henderson, B. E. P., Smith, J. R., Matchett, K. P., Portman, J. R., Wallenborg, K., Picelli, S., Zagorska, A., Pendem, S. V., Hudson, T. E., Wu, M. M., Budas, G. R., Breckenridge, D. G., Harrison, E. M., Mole, D. J., Wigmore, S. J., Ramachandran, P., Ponting, C. P., … Henderson, N. C. (2019). Single-cell transcriptomics uncovers zonation of function in the mesenchyme during liver fibrosis. *Cell Reports*, *29*, 1832–1847.

Drenth, J. P. H., & Schattenberg, J. M. (2020). The nonalcoholic steatohepatitis (NASH) drug development graveyard: Established hurdles and planning for future success. *Expert Opinion on Investigational Drugs*, *29*, 1365–1375.

Ebrahimkhani, M. R., Oakley, F., Murphy, L. B., Mann, J., Moles, A., Perugorria, M. J., Ellis, E., Lakey, A. F., Burt, A. D., Douglass, A., Wright, M. C., White, S. A., Jaffré, F., Maroteaux, L., & Mann, D. A. (2011). Stimulating healthy tissue regeneration by targeting the 5-HT 2B receptor in chronic liver disease. *Nature Medicine*, *17*, 1668–1673.

Ehling, J., Bartneck, M., Wei, X., Gremse, F., Fech, V., Möckel, D., Baeck, C., Hittatiya, K., Eulberg, D., Luedde, T., Kiessling, F., Trautwein, C., Lammers, T., & Tacke, F. (2014). CCL2-dependent infiltrating macrophages promote angiogenesis in progressive liver fibrosis. *Gut*, *63*, 1960–1971.

Ellis, E. L., & Mann, D. A. (2012). Clinical evidence for the regression of liver fibrosis. *Journal of Hepatology*, *56*, 1171–1180.

Elsharkawy, A. M., Oakley, F., & Mann, D. A. (2005). The role and regulation of hepatic stellate cell apoptosis in reversal of liver fibrosis. *Apoptosis*, *10*, 927–939.

Eom, Y. W., Shim, K. Y., & Baik, S. K. (2015). Mesenchymal stem cell therapy for liver fibrosis. *The Korean Journal of Internal Medicine*, *30*, 580.

Esparza-Baquer, A., Labiano, I., Sharif, O., Agirre-Lizaso, A., Oakley, F., Rodrigues, P. M., Zhuravleva, E., O'Rourke, C. J., Hijona, E., Jimenez-Agüero, R., Riaño, I., Landa, A., La Casta, A., Zaki, M. Y. W., Munoz-Garrido, P., Azkargorta, M., Elortza, F., Vogel, A., Schabbauer, G., … Perugorria, M. J. (2021). TREM-2 defends the liver against hepatocellular carcinoma through multifactorial protective mechanisms. *Gut*, *70*, 1345–1361.

Fabris, L., Fiorotto, R., Spirli, C., Cadamuro, M., Mariotti, V., Perugorria, M. J., Banales, J. M., & Strazzabosco, M. (2019). Pathobiology of inherited biliary diseases: A roadmap to understand acquired liver diseases. *Nature Reviews. Gastroenterology & Hepatology*, *16*, 497–511.

Feder, L. S., Todaro, J. A., & Laskin, D. L. (1993). Characterization of interleukin-1 and interleukin-6 production by hepatic endothelial cells and macrophages. *Journal of Leukocyte Biology*, *53*, 126–132.

Ferreira-Gonzalez, S., Lu, W.-Y., Raven, A., Dwyer, B., Man, T. Y., O'Duibhir, E., Starkey Lewis, P. J., Campana, L., Kendall, T. J., Bird, T. G., Tarrats, N., Acosta, J.-C., Boulter, L., & Forbes, S. J. (2018). Paracrine cellular senescence exacerbates biliary injury and impairs regeneration. *Nature Communications*, *9*, 1–15.

Ferreira-Gonzalez, S., Rodrigo-Torres, D., Gadd, V. L., & Forbes, S. J. (2021). Cellular senescence in liver disease and regeneration. In *Seminars in liver disease*. Thieme Medical Publishers, Inc.

Friedman, S. L., Ratziu, V., Harrison, S. A., Abdelmalek, M. F., Aithal, G. P., Caballeria, J., Francque, S., Farrell, G., Kowdley, K. V., Craxi, A., Simon, K., Fischer, L., Melchor-Khan, L., Vest, J., Wiens, B. L., Vig, P., Seyedkazemi, S., Goodman, Z., Wong, V. W.-S., … Lefebvre, E. (2018). A randomized, placebo-controlled trial of cenicriviroc for treatment of nonalcoholic steatohepatitis with fibrosis. *Hepatology*, *67*, 1754–1767.

Garbuzenko, D. V., & Arefyev, N. O. (2017). Hepatic hydrothorax: An update and review of the literature. *World Journal of Hepatology*, *9*, 1197.

Gieling, R. G., Burt, A. D., & Mann, D. A. (2008). Fibrosis and cirrhosis reversibility–molecular mechanisms. *Clinics in Liver Disease*, *12*, 915–937.

Golabi, P., Otgonsuren, M., Cable, R., Felix, S., Koenig, A., Sayiner, M., & Younossi, Z. M. (2016). Non-alcoholic fatty liver disease (NAFLD) is associated with impairment of health related quality of life (HRQOL). *Health and Quality of Life Outcomes*, *14*, 1–7.

Grygiel-Górniak, B. (2014). Peroxisome proliferator-activated receptors and their ligands: Nutritional and clinical implications-a review. *Nutrition Journal*, *13*, 1–10.

Guicciardi, M. E., & Gores, G. J. (2010). Apoptosis as a mechanism for liver disease progression. In *Seminars in liver disease* (pp. 402–410). © Thieme Medical Publishers.

Guicciardi, M. E., Malhi, H., Mott, J. L., & Gores, G. J. (2013). Apoptosis and necrosis in the liver. *Comprehensive Physiology*, *3*, 977–1010.

Hara, A., Niwa, M., Noguchi, K., Kanayama, T., Niwa, A., Matsuo, M., Hatano, Y., & Tomita, H. (2020). Galectin-3 as a next-generation biomarker for detecting early stage of various diseases. *Biomolecules*, *10*, 389.

Harrison, S. A., Bashir, M. R., Lee, K.-J., Shim-Lopez, J., Lee, J., Wagner, B., Smith, N. D., Chen, H. C., & Lawitz, E. J. (2021). A structurally optimized FXR agonist, MET409, reduced liver fat content over 12 weeks in patients with non-alcoholic steatohepatitis. *Journal of Hepatology*, *75*(1), 25–33.

Hong, F., Tuyama, A., Lee, T. F., Loke, J., Agarwal, R., Cheng, X., Garg, A., Fiel, M. I., Schwartz, M., Walewski, J., Branch, A., Schecter, A. D., & Bansal, M. B. (2009). Hepatic stellate cells express functional CXCR4: Role in stromal cell–derived factor-1α–mediated stellate cell activation. *Hepatology*, *49*, 2055–2067.

Hou, J., Zhang, J., Cui, P., Zhou, Y., Liu, C., Wu, X., Ji, Y., Wang, S., Cheng, B., Ye, H., Shu, L., Zhang, K., Di Wang, J. X., Shu, Q., Colonna, M., & Fang, X. (2021). TREM2 sustains macrophage-hepatocyte metabolic coordination in nonalcoholic fatty liver disease and sepsis. *The Journal of Clinical Investigation*, *131*(4), e135197.

Ikeda, K., Wang, L.-H., Torres, R., Zhao, H., Olaso, E., Eng, F. J., Labrador, P., Klein, R., Lovett, D., Yancopoulos, G. D., Friedman, S. L., & Lin, H. C. (2002). Discoidin domain receptor 2 interacts with Src and Shc following its activation by type I collagen. *The Journal of Biological Chemistry*, *277*, 19206–19212.

Ip, E., Farrell, G. C., Robertson, G., Hall, P., Kirsch, R., & Leclercq, I. (2003). Central role of PPARα-dependent hepatic lipid turnover in dietary steatohepatitis in mice. *Hepatology*, *38*, 123–132.

Iwaisako, K., Jiang, C., Zhang, M., Cong, M., Moore-Morris, T. J., Park, T. J., Liu, X., Xu, J., Wang, P., Paik, Y.-H., Meng, F., Asagiri, M., Murray, L. A., Hofmann, A. F., Iida, T., Glass, C. K., Brenner, D. A., & Kisseleva, T. (2014). Origin of myofibroblasts in the fibrotic liver in mice. *Proceedings of the National Academy of Sciences*, *111*, E3297–E3305.

Jain, M. R., Giri, S. R., Bhoi, B., Trivedi, C., Rath, A., Rathod, R., Ranvir, R., Kadam, S., Patel, H., Swain, P., Roy, S. S., Das, N., Karmakar, E., Wahli, W., & Patel, P. R. (2018). Dual PPAR α/γ agonist Saroglitazar improves liver histopathology and biochemistry in experimental NASH models. *Liver International*, *38*, 1084–1094.

Jenne, C. N., & Kubes, P. (2013). Immune surveillance by the liver. *Nature Immunology*, *14*, 996–1006.

Jia, Y., Wang, F., Guo, Q., Li, M., Wang, L., Zhang, Z., Jiang, S., Jin, H., Chen, A., Tan, S., Zhang, F., Shao, J., & Zheng, S. (2018). Curcumol induces RIPK1/RIPK3 complex-dependent necroptosis via JNK1/2-ROS signaling in hepatic stellate cells. *Redox Biology*, *19*, 375–387.

Jiang, W., Tan, Y., Cai, M., Zhao, T., Mao, F., Zhang, X., Xu, W., Yan, Z., Qian, H., & Yan, Y. (2018). Human umbilical cord MSC-derived exosomes suppress the development of CCl4-induced liver injury through antioxidant effect. *Stem Cells International*, *2018*, 6079642.

Karin, D., Koyama, Y., Brenner, D., & Kisseleva, T. (2016). The characteristics of activated portal fibroblasts/myofibroblasts in liver fibrosis. *Differentiation*, *92*, 84–92.

Karlmark, K. R., Weiskirchen, R., Zimmermann, H. W., Gassler, N., Ginhoux, F., Weber, C., Merad, M., Luedde, T., Trautwein, C., & Tacke, F. (2009). Hepatic recruitment of the inflammatory Gr1+ monocyte subset upon liver injury promotes hepatic fibrosis. *Hepatology*, *50*, 261–274.

Karsdal, M. A., Manon-Jensen, T., Genovese, F., Kristensen, J. H., Nielsen, M. J., Sand, J. M. B., Hansen, N.-U. B., Bay-Jensen, A.-C., Bager, C. L., Krag, A., Blanchard, A., Krarup, H., Leeming, D. J., & Schuppan, D. (2015). Novel insights into the function and dynamics of extracellular matrix in liver fibrosis. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *308*, G807–G830.

Kato, T., Ito, Y., Hosono, K., Suzuki, T., Tamaki, H., Minamino, T., Kato, S., Sakagami, H., Shibuya, M., & Majima, M. (2011). Vascular endothelial growth factor receptor-1 signaling promotes liver repair through restoration of liver microvasculature after acetaminophen hepatotoxicity. *Toxicological Sciences*, *120*, 218–229.

Kaul, U., Parmar, D., Manjunath, K., Shah, M., Parmar, K., Patil, K. P., & Jaiswal, A. (2019). New dual peroxisome proliferator activated receptor agonist—Saroglitazar in diabetic dyslipidemia and non-alcoholic fatty liver disease: Integrated analysis of the real world evidence. *Cardiovascular Diabetology*, *18*, 1–11.

Kisseleva, T., & Brenner, D. A. (2007). Role of hepatic stellate cells in fibrogenesis and the reversal of fibrosis. *Journal of Gastroenterology and Hepatology*, *22*, S73–S78.

Kisseleva, T., & Brenner, D. A. (2008). Mechanisms of fibrogenesis. *Experimental Biology and Medicine*, *233*, 109–122.

Kisseleva, T., Cong, M., Paik, Y., Scholten, D., Jiang, C., Benner, C., Iwaisako, K., Moore-Morris, T., Scott, B., Tsukamoto, H., Evans, S. M., Dillmann, W., Glass, C. K., & Brenner, D. A. (2012). Myofibroblasts revert to an inactive phenotype during regression of liver fibrosis. *Proceedings of the National Academy of Sciences*, *109*, 9448–9453.

Kmiec, Z. (2001). Cooperation of liver cells in health and disease: With 18 tables. *Advances in Anatomy, Embryology, and Cell Biology*, *161*, 1–151.

Kremoser, C. (2021). FXR agonists for NASH: How are they different and what difference do they make? *Journal of Hepatology*, *75*(1), 12–15.

Krishna, M. (2017). Patterns of necrosis in liver disease. *Clinics in Liver Disease*, *10*, 53.

Lagnado, A., Leslie, J., Ruchaud-Sparagano, M., Victorelli, S., Hirsova, P., Ogrodnik, M., Collins, A. L., Vizioli, M. G., Habiballa, L., Saretzki, G., Evans, S. A., Salmonowicz, H., Hruby, A., Geh, D., Pavelko, K. D., Dolan, D., Reeves, H. L., Grellscheid, S., Wilson, C. H., … Passos, J. F. (2021). Neutrophils induce paracrine telomere dysfunction and senescence in ROS-dependent manner. *The EMBO Journal*, *40*, e106048.

Lang, A., Schoonhoven, R., Tuvia, S., Brenner, D. A., & Rippe, R. A. (2000). Nuclear factor κB in proliferation, activation, and apoptosis in rat hepatic stellate cells. *Journal of Hepatology*, *33*, 49–58.

Lee, C., Kim, M., Han, J., Yoon, M., & Jung, Y. (2021). Mesenchymal stem cells influence activation of hepatic stellate cells, and constitute a promising therapy for liver fibrosis. *Biomedicine*, *9*, 1598.

Lee, U. E., & Friedman, S. L. (2011). Mechanisms of hepatic fibrogenesis. *Best Practice & Research. Clinical Gastroenterology*, *25*, 195–206.

Lepreux, S., & Desmoulière, A. (2015). Human liver myofibroblasts during development and diseases with a focus on portal (myo)fibroblasts. *Frontiers in Physiology*, *6*, 173.

Leslie, J., Macia, M. G., Luli, S., Worrell, J. C., Reilly, W. J., Paish, H. L., Knox, A., Barksby, B. S., Gee, L. M., Zaki, M. Y. W., Collins, A. L., Burgoyne, R. A., Cameron, R., Bragg, C., Xu, X., Chung, G. W., Brown, C. D. A., Blanchard, A. D., Nanthakumar, C. B., … Oakley, F. (2020). C-Rel orchestrates energy-dependent epithelial and macrophage reprogramming in fibrosis. *Nature Metabolism*, *2*, 1350–1367.

Li, H., Zheng, S., Liu, F., Liu, W., & Zhao, R. (2021). Fighting against COVID-19: Innovative strategies for clinical pharmacists. *Research in Social and Administrative Pharmacy*, *17*, 1813–1818.

Li, T., Yan, Y., Wang, B., Qian, H., Zhang, X., Shen, L., Wang, M., Zhou, Y., Zhu, W., Li, W., & Xu, W. (2013). Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis. *Stem Cells and Development*, *22*, 845–854.

Liu, X., Xu, J., Rosenthal, S., Zhang, L., McCubbin, R., Meshgin, N., Shang, L., Koyama, Y., Ma, H.-Y., Sharma, S., Heinz, S., Glass, C. K., Benner, C., Brenner, D. A., & Kisseleva, T. (2020). Identification of lineage-specific transcription factors that prevent activation of hepatic stellate cells and promote fibrosis resolution. *Gastroenterology*, *158*, 1728–1744.

Ma, H., Liu, X., Zhang, M., & Niu, J. (2021). Liver sinusoidal endothelial cells are implicated in multiple fibrotic mechanisms. *Molecular Biology Reports*, *48*, 2803–2815.

Macdonald, G. A., & Prins, J. B. (2004). Peroxisomal fatty acid metabolism, peroxisomal proliferator-activated receptors and non-alcoholic fatty liver disease. *Journal of Gastroenterology and Hepatology*, *19*, 1335–1337.

Maher, J. J., Lozier, J. S., & Scott, M. K. (1998). Rat hepatic stellate cells produce cytokine-induced neutrophil chemoattractant in culture and in vivo. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *275*, G847–G853.

Maiers, J. L., & Malhi, H. (2019). Endoplasmic reticulum stress in metabolic liver diseases and hepatic fibrosis. *Seminars in Liver Disease*, *39*, 235–248.

Mann, J., & Mann, D. A. (2009). Transcriptional regulation of hepatic stellate cells. *Advanced Drug Delivery Reviews*, *61*, 497–512.

Marcellin, P., Gane, E., Buti, M., Afdhal, N., Sievert, W., Jacobson, I. M., Washington, M. K., Germanidis, G., Flaherty, J. F., Schall, R. A., Bornstein, J. D., Kitrinos, K. M., Subramanian, G. M., McHutchison, J. G., & Heathcote, E. J. (2013). Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: A 5-year open-label follow-up study. *Lancet*, *381*, 468–475.

Marra, F., DeFranco, R., Grappone, C., Milani, S., Pastacaldi, S., Pinzani, M., Romanelli, R. G., & Laffi, G. (1998). Increased expression of monocyte chemotactic protein-1 during active hepatic fibrogenesis: Correlation with monocyte infiltration. *The American Journal of Pathology*, *152*, 423.

Marra, F., Valente, A. J., Pinzani, M., & Abboud, H. E. (1993). Cultured human liver fat-storing cells produce monocyte chemotactic protein-1. Regulation by proinflammatory cytokines. *The Journal of Clinical Investigation*, *92*, 1674–1680.

Martin, K., Pritchett, J., Llewellyn, J., Mullan, A. F., Athwal, V. S., Dobie, R., Harvey, E., Zeef, L., Farrow, S., Streuli, C., Henderson, N. C., Friedman, S. L., Hanley, N. A., & Hanley, K. P. (2016). PAK proteins and YAP-1 signalling downstream of integrin beta-1 in myofibroblasts promote liver fibrosis. *Nature Communications*, *7*, 1–11.

Mazzarelli, C., Angrisani, D., & Vigano, R. (2020). The rising tide of non-alcoholic fatty liver disease: How much time until the flood reaches our coast? *Clinical Gastroenterology and Hepatology*, *19*(6), 1299.

Mederacke, I., Hsu, C. C., Troeger, J. S., Huebener, P., Mu, X., Dapito, D. H., Pradere, J.-P., & Schwabe, R. F. (2013). Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. *Nature Communications*, *4*, 1–11.

Meng, F., Wang, K., Aoyama, T., Grivennikov, S. I., Paik, Y., Scholten, D., Cong, M., Iwaisako, K., Liu, X., Zhang, M., Österreicher, C. H., Stickel, F., Ley, K., Brenner, D. A., & Kisseleva, T. (2012). Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. *Gastroenterology*, *143*, 765–776.

Michalopoulos, G. K., & DeFrances, M. C. (1997). Liver regeneration. *Science*, *276*, 60–66.

Miura, K., Yang, L., van Rooijen, N., Ohnishi, H., & Seki, E. (2012). Hepatic recruitment of macrophages promotes nonalcoholic steatohepatitis through CCR2. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *302*, G1310–G1321.

Miyahara, T., Schrum, L., Rippe, R., Xiong, S., Yee, H. F., Motomura, K., Anania, F. A., Willson, T. M., & Tsukamoto, H. (2000). Peroxisome proliferator-activated receptors and hepatic stellate cell activation. *The Journal of Biological Chemistry*, *275*, 35715–35722.

Moon, A. M., Singal, A. G., & Tapper, E. B. (2020). Contemporary epidemiology of chronic liver disease and cirrhosis. *Clinical Gastroenterology and Hepatology*, *18*, 2650–2666.

Moroni, F., Dwyer, B. J., Graham, C., Pass, C., Bailey, L., Ritchie, L., Mitchell, D., Glover, A., Laurie, A., Doig, S., Hargreaves, E., Fraser, A. R., Turner, M. L., Campbell, J. D. M., McGowan, N. W. A., Barry, J., Moore, J. K., Hayes, P. C., Leeming, D. J., … Forbes, S. J. (2019). Safety profile of autologous macrophage therapy for liver cirrhosis. *Nature Medicine*, *25*, 1560–1565.

Mudaliar, S., Henry, R. R., Sanyal, A. J., Morrow, L., Marschall, H., Kipnes, M., Adorini, L., Sciacca, C. I., Clopton, P., Castelloe, E., Dillon, P., Pruzanski, M., & Shapiro, D. (2013). Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. *Gastroenterology*, *145*, 574–582.

Musso, G., Cassader, M., & Gambino, R. (2016). Non-alcoholic steatohepatitis: Emerging molecular targets and therapeutic strategies. *Nature Reviews. Drug Discovery*, *15*, 249–274.

Nakamoto, N., Ebinuma, H., Kanai, T., Chu, P., Ono, Y., Mikami, Y., Ojiro, K., Lipp, M., Love, P. E., Saito, H., & Hibi, T. (2012). CCR9+ macrophages are required for acute liver inflammation in mouse models of hepatitis. *Gastroenterology*, *142*, 366–376.

Nakano, Y., Kamiya, A., Sumiyoshi, H., Tsuruya, K., Kagawa, T., & Inagaki, Y. (2020). A deactivation factor of fibrogenic hepatic stellate cells induces regression of liver fibrosis in mice. *Hepatology*, *71*, 1437–1452.

Nanji, A. A., & Hiller-Sturmhöfel, S. (1997). Apoptosis and necrosis: Two types of cell death in alcoholic liver disease. *Alcohol Health and Research World*, *21*, 325.

Neuschwander-Tetri, B. A., Loomba, R., Sanyal, A. J., Lavine, J. E., Van Natta, M. L., Abdelmalek, M. F., Chalasani, N., Dasarathy, S., Diehl, A. M., Hameed, B., Kowdley, K. V., McCullough, A., Terrault, N., Clark, J. M., Tonascia, J., Brunt, E. M., Kleiner, D. E., Doo, E., & NASH Clinical Research Network. (2015). Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): A multicentre, randomised, placebo-controlled trial. *Lancet*, *385*, 956–965.

Ni, X.-X., Li, X.-Y., Wang, Q., & Hua, J. (2021). Regulation of peroxisome proliferator-activated receptor-gamma activity affects the hepatic stellate cell activation and the progression of NASH via TGF-β1/Smad signaling pathway. *Journal of Physiology and Biochemistry*, *77*, 35–45.

Niu, Z., Wang, M., Zhou, L., Yao, L., Liao, Q., & Zhao, Y. (2015). Elevated GRP78 expression is associated with poor prognosis in patients with pancreatic cancer. *Scientific Reports*, *5*, 1–12.

Noureddin, M., & Sanyal, A. J. (2018). Pathogenesis of NASH: The impact of multiple pathways. *Current Hepatology Reports*, *17*, 350–360.

Oakley, F., Meso, M., Iredale, J. P., Green, K., Marek, C. J., Zhou, X., May, M. J., Millward-Sadler, H., Wright, M. C., & Mann, D. A. (2005). Inhibition of inhibitor of κB kinases stimulates hepatic stellate cell apoptosis and accelerated recovery from rat liver fibrosis. *Gastroenterology*, *128*, 108–120.

Ogrodnik, M., Miwa, S., Tchkonia, T., Tiniakos, D., Wilson, C. L., Lahat, A., Day, C. P., Burt, A., Palmer, A., Anstee, Q. M., Grellscheid, S. N., Hoeijmakers, J. H. J., Barnhoorn, S., Mann, D. A., Bird, T. G., Vermeij, W. P., Kirkland, J. L., Passos, J. F., von Zglinicki, T., & Jurk, D. (2017). Cellular senescence drives age-dependent hepatic steatosis. *Nature Communications*, *8*, 1–12.

Olaso, E. (1999). The discoidin domain receptor 2 (DDR2) is induced during stellate cell activation, mediates cell growth and MMP2 expression, and is regulated by extracellular matrix. *Hepatology*, *30*, 413A.

Olaso, E., Ikeda, K., Eng, F. J., Xu, L., Wang, L.-H., Lin, H. C., & Friedman, S. L. (2001). DDR2 receptor promotes MMP-2–mediated proliferation and invasion by hepatic stellate cells. *The Journal of Clinical Investigation*, *108*, 1369–1378.

Page, A., Paoli, P., Salvador, E. M., White, S., French, J., & Mann, J. (2016). Hepatic stellate cell transdifferentiation involves genome-wide remodeling of the DNA methylation landscape. *Journal of Hepatology*, *64*, 661–673.

Paish, H. L., Reed, L. H., Brown, H., Bryan, M. C., Govaere, O., Leslie, J., Barksby, B. S., Macia, M. G., Watson, A., Xu, X., Zaki, M. Y. W., Greaves, L., Whitehall, J., French, J., White, S. A., Manas, D. M., Robinson, S. M., Spoletini, G., Griffiths, C., … Oakley, F. (2019). A bioreactor technology for modeling fibrosis in human and rodent precision-cut liver slices. *Hepatology*, *70*, 1377–1391.

Parkes, J., Guha, I. N., Roderick, P., Harris, S., Cross, R., Manos, M. M., Irving, W., Zaitoun, A., Wheatley, M., Ryder, S., & Rosenberg, W. (2011). Enhanced liver fibrosis (ELF) test accurately identifies liver fibrosis in patients with chronic hepatitis C. *Journal of Viral Hepatitis*, *18*, 23–31.

Parsons, C. J., Bradford, B. U., Pan, C. Q., Cheung, E., Schauer, M., Knorr, A., Krebs, B., Kraft, S., Zahn, S., Brocks, B., Feirt, N., Mei, B., Cho, M.-S., Ramamoorthi, R., Roldan, G., Ng, P., Lum, P., Hirth-Dietrich, C., Tomkinson, A., & Brenner, D. A. (2004). Antifibrotic effects of a tissue inhibitor of metalloproteinase-1 antibody on established liver fibrosis in rats. *Hepatology*, *40*, 1106–1115.

Pawlak, M., Lefebvre, P., & Staels, B. (2015). Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. *Journal of Hepatology*, *62*, 720–733.

Pellicoro, A., Ramachandran, P., Iredale, J. P., & Fallowfield, J. A. (2014). Liver fibrosis and repair: Immune regulation of wound healing in a solid organ. *Nature Reviews. Immunology*, *14*, 181–194.

Perugorria, M. J., Esparza-Baquer, A., Oakley, F., Labiano, I., Korosec, A., Jais, A., Mann, J., Tiniakos, D., Santos-Laso, A., Arbelaiz, A., Gawish, R., Sampedro, A., Fontanellas, A., Hijona, E., Jimenez-Agüero, R., Esterbauer, H., Stoiber, D., Bujanda, L., Banales, J. M., … Mann, D. A. (2019). Non-parenchymal TREM-2 protects the liver from immune-mediated hepatocellular damage. *Gut*, *68*, 533–546.

Perugorria, M. J., Wilson, C. L., Zeybel, M., Walsh, M., Amin, S., Robinson, S., White, S. A., Burt, A. D., Oakley, F., Tsukamoto, H., Mann, D. A., & Mann, J. (2012). Histone methyltransferase ASH1 orchestrates fibrogenic gene transcription during myofibroblast transdifferentiation. *Hepatology*, *56*, 1129–1139.

Pierre, B., Elisabetta, B., Vlad, R., Philippe, H.-M., Bruno, S., Jean-Louis, J., Pierre, B., & Jean-Louis, A. (2020). A randomised, double-blind, placebo-controlled, multi-Centre, dose-range, proof-of-concept, 24-week treatment study of lanifibranor in adult subjects with non-alcoholic steatohepatitis: Design of the NATIVE study. *Contemporary Clinical Trials*, *98*, 106170.

Pugliese, G., Iacobini, C., Pesce, C. M., & Menini, S. (2015). Galectin-3: An emerging all-out player in metabolic disorders and their complications. *Glycobiology*, *25*, 136–150.

Quaglia, A., Alves, V. A., Balabaud, C., Bhathal, P. S., Bioulac-Sage, P., Crawford, J. M., Dhillon, A. P., Ferrell, L., Guido, M., Hytiroglou, P., Nakanuma, Y., Paradis, V., Snover, D. C., Theise, N. D., Thung, S. N., Tsui, W. M. S., van Leeuwen, D. J., & The International Liver Pathology Study Group. (2016). Role of aetiology in the progression, regression, and parenchymal remodelling of liver disease: Implications for liver biopsy interpretation. *Histopathology*, *68*, 953–967. https://doi.org/10.1111/his.12957

Quante, M., Tu, S. P., Tomita, H., Gonda, T., Wang, S. S. W., Takashi, S., Baik, G. H., Shibata, W., Diprete, B., Betz, K. S., Friedman, R., Varro, A., Tycko, B., & Wang, T. C. (2011). Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. *Cancer Cell*, *19*, 257–272.

Ramachandran, P., Dobie, R., Wilson-Kanamori, J. R., Dora, E. F., Henderson, B. E. P., Luu, N. T., Portman, J. R., Matchett, K. P., Brice, M., Marwick, J. A., Taylor, R. S., Efremova, M., Vento-Tormo, R., Carragher, N. O., Kendall, T. J., Fallowfield, J. A., Harrison, E. M., Mole, D. J., Wigmore, S. J., … Henderson, N. C. (2019). Resolving the fibrotic niche of human liver cirrhosis at single-cell level. *Nature*, *575*, 512–518.

Ramachandran, P., Pellicoro, A., Vernon, M. A., Boulter, L., Aucott, R. L., Ali, A., Hartland, S. N., Snowdon, V. K., Cappon, A., Gordon-Walker, T. T., Williams, M. J., Dunbar, D. R., Manning, J. R., van Rooijen, N., Fallowfield, J. A., Forbes, S. J., & Iredale, J. P. (2012). Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis. *Proceedings of the National Academy of Sciences*, *109*, E3186–E3195.

Ratziu, V., Sanyal, A., Harrison, S. A., Wong, V. W., Francque, S., Goodman, Z., Aithal, G. P., Kowdley, K. V., Seyedkazemi, S., Fischer, L., Loomba, R., Abdelmalek, M. F., & Tacke, F. (2020). Cenicriviroc treatment for adults with nonalcoholic steatohepatitis and fibrosis: Final analysis of the phase 2b CENTAUR study. *Hepatology*, *72*, 892–905.

Ribeiro, P. S., Cortez-Pinto, H., Solá, S., Castro, R. E., Ramalho, R. M., Baptista, A., Moura, M. C., Camilo, M. E., & Rodrigues, C. M. P. (2004). Hepatocyte apoptosis, expression of death receptors, and activation of NF-κ B in the liver of nonalcoholic and alcoholic steatohepatitis patients. *The American Journal of Gastroenterology*, *99*, 1708–1717.

Rockey, D. C. (2019). Fibrosis reversal after hepatitic C virus elimination. *Current Opinion in Gastroenterology*, *35*, 137.

Rong, X., Li, J., Yang, Y., Shi, L., & Jiang, T. (2019). Human fetal skin-derived stem cell secretome enhances radiation-induced skin injury therapeutic effects by promoting angiogenesis. *Stem Cell Research & Therapy*, *10*, 1–11.

Rudolph, K. L., Chang, S., Millard, M., Schreiber-Agus, N., & DePinho, R. A. (2000). Inhibition of experimental liver cirrhosis in mice by telomerase gene delivery. *Science*, *287*, 1253–1258.

Sasaki, R., Devhare, P., Ray, R. B., & Ray, R. (2017). Hepatitis C virus–induced tumor-initiating cancer stem–like cells activate stromal fibroblasts in a xenograft tumor model. *Hepatology*, *66*, 1766–1778.

Sato, K., Marzioni, M., Meng, F., Francis, H., Glaser, S., & Alpini, G. (2019). Ductular reaction in liver diseases: Pathological mechanisms and translational significances. *Hepatology*, *69*, 420–430.

Schattenberg, J. M., Nagel, M., Kim, Y. O., Kohl, T., Wörns, M. A., Zimmermann, T., Schad, A., Longerich, T., Schuppan, D., He, Y.-W., Galle, P. R., & Schuchmann, M. (2012). Increased hepatic fibrosis and JNK2-dependent liver injury in mice exhibiting hepatocyte-specific deletion of cFLIP. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *303*, G498–G506.

Schuppan, D., & Afdhal, N. H. (2008). Liver cirrhosis. *Lancet (London, England)*, *371*, 838–851.

Schuppan, D., Ashfaq-Khan, M., Yang, A. T., & Kim, Y. O. (2018). Liver fibrosis: Direct antifibrotic agents and targeted therapies. *Matrix Biology*, *68*, 435–451.

Schwabe, R. F., & Luedde, T. (2018). Apoptosis and necroptosis in the liver: A matter of life and death. *Nature Reviews. Gastroenterology & Hepatology*, *15*, 738–752.

Sepanlou, S. G., Safiri, S., Bisignano, C., Ikuta, K. S., Merat, S., Saberifiroozi, M., Poustchi, H., Tsoi, D., Colombara, D. V., Abdoli, A., Adedoyin, R. A., Afarideh, M., Agrawal, S., Ahmad, S., Ahmadian, E., Ahmadpour, E., Akinyemiju, T., Akunna, C. J., Alipour, V., … Malekzadeh, R. (2020). The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990–2017: A systematic analysis for the global burden of disease study 2017. *The Lancet Gastroenterology & Hepatology*, *5*, 245–266.

Shetty, S., Bruns, T., Weston, C. J., Stamataki, Z., Oo, Y. H., Long, H. M., Reynolds, G. M., Pratt, G., Moss, P., Jalkanen, S., & Hubscher, S. G. (2012). Recruitment mechanisms of primary and malignant B cells to the human liver. *Hepatology*, *56*, 1521–1531.

Singh, S., Allen, A. M., Wang, Z., Prokop, L. J., Murad, M. H., & Loomba, R. (2015). Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: A systematic review and meta-analysis of paired-biopsy studies. *Clinical Gastroenterology and Hepatology*, *13*, 643–654.

Sirica, A. E., Gores, G. J., Groopman, J. D., Selaru, F. M., Strazzabosco, M., Wei Wang, X., & Zhu, A. (2019). Intrahepatic cholangiocarcinoma: Continuing challenges and translational advances. *Hepatology*, *69*, 1803–1815.

Soustiel, F. J., & Zaaroor, M. (2012). Mitochondrial targeting for development of novel drug strategies in brain injury. *Central Nervous System Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Central Nervous System Agents)*, *12*, 131–145.

Suarez-Cuenca, J. A., Chagoya de Sanchez, V., Aranda-Fraustro, A., Sanchez-Sevilla, L., Martinez-Perez, L., & Hernandez-Munoz, R. (2008). Partial hepatectomy–induced regeneration accelerates reversion of liver fibrosis involving participation of hepatic stellate cells. *Experimental Biology and Medicine*, *233*, 827–839.

Tacke, F. (2012). Functional role of intrahepatic monocyte subsets for the progression of liver inflammation and liver fibrosis in vivo. In *Fibrogenesis & tissue repair* (pp. 1–8). Springer.

Tan, Z., Qian, X., Jiang, R., Liu, Q., Wang, Y., Chen, C., Wang, X., Ryffel, B., & Sun, B. (2013). IL-17A plays a critical role in the pathogenesis of liver fibrosis through hepatic stellate cell activation. *Journal of Immunology*, *191*, 1835–1844.

Thiele, N. D., Wirth, J. W., Steins, D., Koop, A. C., Ittrich, H., Lohse, A. W., & Kluwe, J. (2017). TIMP-1 is upregulated, but not essential in hepatic fibrogenesis and carcinogenesis in mice. *Scientific Reports*, *7*, 1–9.

Traber, P. G., & Zomer, E. (2013). Therapy of experimental NASH and fibrosis with galectin inhibitors. *PLoS ONE*, *8*, e83481.

Trautwein, C., Friedman, S. L., Schuppan, D., & Pinzani, M. (2015). Hepatic fibrosis: Concept to treatment. *Journal of Hepatology*, *62*, S15–S24.

Trivedi, P., Wang, S., & Friedman, S. L. (2020). The power of plasticity—Metabolic regulation of hepatic stellate cells. *Cell Metabolism*.

Troeger, J. S., Mederacke, I., Gwak, G., Dapito, D. H., Mu, X., Hsu, C. C., Pradere, J.-P., Friedman, R. A., & Schwabe, R. F. (2012). Deactivation of hepatic stellate cells during liver fibrosis resolution in mice. *Gastroenterology*, *143*, 1073–1083.

Tsuchida, T., & Friedman, S. L. (2017). Mechanisms of hepatic stellate cell activation. *Nature Reviews. Gastroenterology & Hepatology*, *14*, 397.

Tsukuma, H., Hiyama, T., Tanaka, S., Nakao, M., Yabuuchi, T., Kitamura, T., Nakanishi, K., Fujimoto, I., Inoue, A., Yamazaki, H., & Kawashima, T. (1993). Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *The New England Journal of Medicine*, *328*, 1797–1801.

Von Bahr, L., Batsis, I., Moll, G., Hägg, M., Szakos, A., Sundberg, B., Uzunel, M., Ringden, O., & Le Blanc, K. (2012). Analysis of tissues following mesenchymal stromal cell therapy in humans indicates limited long-term engraftment and no ectopic tissue formation. *Stem Cells*, *30*, 1575–1578.

Vuppalanchi, R., Noureddin, M., Alkhouri, N., & Sanyal, A. J. (2021). Therapeutic pipeline in nonalcoholic steatohepatitis. *Nature Reviews. Gastroenterology & Hepatology*, *18*, 373–392.

Wang, R., Ding, Q., Yaqoob, U., de Assuncao, T. M., Verma, V. K., Hirsova, P., Cao, S., Mukhopadhyay, D., Huebert, R. C., & Shah, V. H. (2015). Exosome adherence and internalization by hepatic stellate cells triggers sphingosine 1-phosphate-dependent migration. *The Journal of Biological Chemistry*, *290*, 30684–30696.

Wehr, C., Gennery, A. R., Lindemans, C., Schulz, A., Hoenig, M., Marks, R., Recher, M., Gruhn, B., Holbro, A., Heijnen, I., Meyer, D., Grigoleit, G., Einsele, H., Baumann, U., Witte, T., Sykora, K.-W., Goldacker, S., Regairaz, L., Aksoylar, S., … Inborn Errors Working Party of the European Society for Blood and Marrow Transplantation and the European Society for Immunodeficiency. (2015). Multicenter experience in hematopoietic stem cell transplantation for serious complications of common variable immunodeficiency. *The Journal of Allergy and Clinical Immunology*, *135*, 988–997.

Wen, Y., Lambrecht, J., Ju, C., & Tacke, F. (2021). Hepatic macrophages in liver homeostasis and diseases-diversity, plasticity and therapeutic opportunities. *Cellular & Molecular Immunology*, *18*, 45–56.

Weng, H., Mertens, P. R., Gressner, A. M., & Dooley, S. (2007). IFN-γ abrogates profibrogenic TGF-β signaling in liver by targeting expression of inhibitory and receptor Smads. *Journal of Hepatology*, *46*, 295–303.

Williams, G. M., & Iatropoulos, M. J. (2002). Alteration of liver cell function and proliferation: Differentiation between adaptation and toxicity. *Toxicologic Pathology*, *30*, 41–53.

Wilson, C. L., Mann, D. A., & Borthwick, L. A. (2017). Epigenetic reprogramming in liver fibrosis and cancer. *Advanced Drug Delivery Reviews*, *121*, 124–132.

Wright, M. C., Issa, R., Smart, D. E., Trim, N., Murray, G. I., Primrose, J. N., Arthur, M. J., Iredale, J. P., & Mann, D. A. (2001). Gliotoxin stimulates the apoptosis of human and rat hepatic stellate cells and enhances the resolution of liver fibrosis in rats. *Gastroenterology*, *121*, 685–698.

Wynn, T. A. (2008). Cellular and molecular mechanisms of fibrosis. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, *214*, 199–210.

Xie, G., Wang, X., Wang, L., Wang, L., Atkinson, R. D., Kanel, G. C., Gaarde, W. A., & Deleve, L. D. (2012). Role of differentiation of liver sinusoidal endothelial cells in progression and regression of hepatic fibrosis in rats. *Gastroenterology*, *142*, 918–927.

Xie, Q., Zhou, X., Huang, P., Wei, J., Wang, W., & Zheng, S. (2014). The performance of enhanced liver fibrosis (ELF) test for the staging of liver fibrosis: A meta-analysis. *PLoS ONE*, *9*, e92772.

Xiong, X., Kuang, H., Ansari, S., Liu, T., Gong, J., Wang, S., Zhao, X.-Y., Ji, Y., Li, C., Guo, L., Zhou, L., Chen, Z., Leon-Mimila, P., Chung, M. T., Kurabayashi, K., Opp, J., Campos-Pérez, F., Villamil-Ramírez, H., Canizales-Quinteros, S., … Lin, J. D. (2019). Landscape of intercellular crosstalk in healthy and NASH liver revealed by single-cell secretome gene analysis. *Molecular Cell*, *75*, 644–660.

Yang, J. D., Hainaut, P., Gores, G. J., Amadou, A., Plymoth, A., & Roberts, L. R. (2019). A global view of hepatocellular carcinoma: Trends, risk, prevention and management. *Nature Reviews. Gastroenterology & Hepatology*, *16*, 589–604.

Yang, L., Chan, C.-C., Kwon, O.-S., Liu, S., McGhee, J., Stimpson, S. A., Chen, L. Z., Harrington, W. W., Symonds, W. T., & Rockey, D. C. (2006). Regulation of peroxisome proliferator-activated receptor-γ in liver fibrosis. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *291*, G902–G911.

Ying, H., Chen, Q., Zhang, W., Zhang, H., Ma, Y., Zhang, S., Fang, J., & Yu, C.-H. (2017). PDGF signaling pathway in hepatic fibrosis pathogenesis and therapeutics. *Molecular Medicine Reports*, *16*, 7879–7889.

Yosef, R., Pilpel, N., Papismadov, N., Gal, H., Ovadya, Y., Vadai, E., Miller, S., Porat, Z., Ben-Dor, S., & Krizhanovsky, V. (2017). p21 maintains senescent cell viability under persistent DNA damage response by restraining JNK and caspase signaling. *The EMBO Journal*, *36*, 2280–2295.

Yoshiji, H., Kuriyama, S., Miyamoto, Y., Thorgeirsson, U. P., Gomez, D. E., Kawata, M., Yoshii, J., Ikenaka, Y., Noguchi, R., Tsujinoue, H., Nakatani, T., Thorgeirsson, S. S., & Fukui, H. (2000). Tissue inhibitor of metalloproteinases-1 promotes liver fibrosis development in a transgenic mouse model. *Hepatology*, *32*, 1248–1254.

Yoshiji, H., Kuriyama, S., Yoshii, J., Ikenaka, Y., Noguchi, R., Nakatani, T., Tsujinoue, H., Yanase, K., Namisaki, T., Imazu, H., & Fukui, H. (2002). Tissue inhibitor of metalloproteinases-1 attenuates spontaneous liver fibrosis resolution in the transgenic mouse. *Hepatology*, *36*, 850–860.

Younossi, Z. M., Ratziu, V., Loomba, R., Rinella, M., Anstee, Q. M., Goodman, Z., Bedossa, P., Geier, A., Beckebaum, S., Newsome, P. N., Sheridan, D., Sheikh, M. Y., Trotter, J., Knapple, W., Lawitz, E., Abdelmalek, M. F., Kowdley, K. V., Montano-Loza, A. J., Boursier, J., … REGENERATE Study Investigators. (2019). Obeticholic acid for the treatment of non-alcoholic steatohepatitis: Interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet*, *394*, 2184–2196.

Zeybel, M., Luli, S., Sabater, L., Hardy, T., Oakley, F., Leslie, J., Page, A., Salvador, E. M., Sharkey, V., Tsukamoto, H., Chu, D. C. K., Singh, U. S., Ponzoni, M., Perri, P., Di Paolo, D., Mendivil, E. J., Mann, J., & Mann, D. A. (2017). A proof-of-concept for epigenetic therapy of tissue fibrosis: Inhibition of liver fibrosis progression by 3-deazaneplanocin a. *Molecular Therapy*, *25*, 218–231.

Zhang, J., Zhang, K., Li, Z., & Guo, B. (2016). ER stress-induced inflammasome activation contributes to hepatic inflammation and steatosis. *Journal of Clinical & Cellular Immunology*, *7*, 457.

Zhang, M., Serna-Salas, S., Damba, T., Borghesan, M., Demaria, M., & Moshage, H. (2021). Hepatic stellate cell senescence in liver fibrosis: Characteristics, mechanisms and perspectives. *Mechanisms of Ageing and Development*, *119*, 111572.

Zingarelli, B., Piraino, G., Hake, P. W., O'Connor, M., Denenberg, A., Fan, H., & Cook, J. A. (2010). Peroxisome proliferator-activated receptor δ regulates inflammation via NF-κB signaling in polymicrobial sepsis. *The American Journal of Pathology*, *177*, 1834–1847.

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