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Early to Phase II drugs currently under investigation for the treatment of liver fibrosis

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Introduction: Chronic liver diseases represent a high unmet medical need and are characterized by persistent inflammation, parenchymal damage and fibrotic remodeling, leading eventually to cirrhosis and hepatic failure. Besides the persisting high prevalence of chronic viral hepatitis B and C, the dramatic increase in nonalcoholic steatohepatitis is now considered to be a major pathophysiologic driver for fibrosis development and subsequently cirrhosis. Increasing evidence suggests that also liver cirrhosis can regress when treated adequately.

Areas covered: Herein, the authors review the underlying pathophysiologic mechanisms leading to fibrotic remodeling in the liver. They also highlight the options for novel treatment strategies by using molecular targeted agents.

Expert opinion: New in vitro and preclinical animal models, and the careful selection of patients with high disease dynamics for clinical studies, provide a sound basis for the clinical development of antifibrotic agents in humans. Surrogate parameters of liver function, inflammation, tissue remodeling and damage, as well as noninvasive imaging techniques, can be applied in clinical trials to provide fast readouts and novel and reliable endpoints for trial design, and provide an attractive regulatory environment for this emerging disease area.

Keywords: cirrhosis, clinical trials, end-stage liver disease, liver fibrosis, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis


1. Introduction

The liver is the central organ involved in a plethora of synthesis, metabolism and detoxification processes in the body. Exposure to chemical agents, toxins or nutrients (e.g., acetaminophen, ethanol), infectious agents (e.g., viral hepatitis) or metabolic conditions (e.g., Wilson’s disease, hemochromatosis) as well as to conditions affecting physiologic bile formation and flow (e.g., primary biliary cirrhosis [PBC], primary sclerosing cholangitis) or autoimmune diseases triggers acute and chronic inflammatory responses in the liver.

Repeated and chronic liver injury by any of these agents leads to hepatocyte damage and is a direct cause of the numerous clinical symptoms like jaundice, ascites, varices, bleeding disorders and encephalopathy in affected patients [1]. Hepatocyte damage triggers the pathophysiologic cascades associated with inflammation and collagen deposition that lead to tissue remodeling and finally cirrhosis development and end-stage liver disease (see below) [2].

The overall epidemiology of fibrotic liver diseases is not well known. Its prevalence is estimated between 4.5 and 9.5% in autopsy studies but is also considered to be largely underestimated due to the silent clinical course and high regenerative capacity of the liver to compensate for potential damages and loss of functional hepatocyte masses [3-5]. A recent study from the UK reported a steep increase in
Liver fibrosis and its end-stage liver cirrhosis thus represent a high medical need. Although some progress has been made in understanding the underlying pathophysiology of matrix deposition and inflammation, treatment options for established fibrotic or cirrhotic liver diseases are still limited giving a dismal prognosis to patients unless a curative approach to the underlying etiology can be achieved [5]. Here, we will review the recent advances in pathophysiologic models, with a special focus on NASH, and their translational value for developing novel pharmacologic therapy options for fibrotic liver diseases. Agents currently under clinical investigation and potential new trial designs and trial endpoints will be highlighted that could lead to better treatment options for affected patients in the future.

2. Pathophysiology of liver fibrosis

The liver responds to injuries and damages with a uniform pattern of hepatocyte degeneration and cell death, which then triggers inflammatory and regenerative programs aiming to compensate for the loss of functional hepatocytes and to restrict parenchymal damage. Persistent injury finally leads to chronic activation of a wound-healing process, which is morphologically characterized by the increased production of extracellular matrix (ECM) components, formation of fibrous septae, parenchymal (regenerative) nodules and consequently the disruption of liver architecture [16-18].

The main source for ECM production is myofibroblast-like cells [15,19] that most commonly trans-differentiate upon hepatic damage from either hepatic stellate cells (HSCs) [2,20,21], or to a lesser extent from portal fibroblasts [22], epithelial cell types [23] or bone marrow–derived mesenchymal stem cells [24-26]. ECM deposition by myofibroblast-like cells occurs in the sub-sinusoidal space of Disse, which physiologically separates hepatocytes from fenestrated hepatic endothelial cells and allows for intensive metabolic exchange due to low-density ECM composition [2]. During fibrogenesis, a quantitative and qualitative change in ECM content of the space of Disse occurs [27], which closes endothelial cell fenestration (capilarization [28]) and impairs the exchange process between the portal venous system and hepatocytes, which finally contributes to the development of portal hypertension, ascites and varices [1,2].

Matrix production by activated HSCs and other myofibroblast-like cells is mediated by a great number of profibrogenic mediators, mainly cytokines and growth factors that are released in a paracrine or autocrine manner from various cell types like T cells, hepatocytes, biliary cells or Kupffer cells [29,30]. Besides activating ECM production, profibrogenic mediators also increase proliferation and inhibit apoptosis of the target cell populations and have chemotactic and inflammatory effects [2,31]. A prominent member of these mediators is TGFβ[1]. Ligand binding leads to dimerization of its cognate receptor on HSCs and activates a signaling cascade via SMAD proteins to regulate gene transcription associated with ECM production [32-34]. Other inflammatory cytokines are various ILs (e.g., IL-1β [35-37], TNFα [38,39], IL-17 [40] or IL-22 [41]) as well as several chemokines like CCL2 (MCP-1) [42,43] or CCL5 (RANTES) [44,45] and toll-like receptors (TLRs), which could either be activated (e.g., TLR4 [46] or TLR9 [47]) or inhibited (e.g., TLR3 [48] and TLR7 [49]) to promote fibrotic processes. A potential key target for drug development could be the lysyl oxidase (LOX) family, where especially lysyl-oxidase like 2 (LOXL2) has been shown to crosslink ECM proteins like collagen and elastin during wound healing, scar formation and fibrogenesis in various organs [50].

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the liver, HSCs and portal fibroblasts, but also hepatocytes, have been identified to be the major sources for LOX proteins and a significant overexpression was observed under fibrotic conditions [51,52]. Suppression of LOX proteins by either miRNA-mediated downregulation or using an inhibitory antibody reduced TGFβ signaling and fibroblast activation, confirming LOX proteins and TGFβ signaling as potential core pathways for antifibrotic drug development [53,54].

Recent experimental data also demonstrate that activated myofibroblasts can be reverted to an inactive phenotype when the causative activating agent is removed, which is then paralleled by regression of fibrosis [55,56]. Thus, targeting myofibroblasts could provide an attractive alternative target for drug development and, for example, inhibition of Notch or Hedgehog signaling in myofibroblasts leads to mesenchymal-to-epithelial like transdifferentiation with reduced fibrosis in animal models [57]. Besides inhibition of myofibroblast activation or induction of apoptosis, also the induction of senescence by, for example, atorvastatin or attenuation of TGFβ via the secreted protein CCN1/CYR61 in these cells has been linked to an antifibrotic effect [58-60].

The interplay between HSCs, hepatocytes and macrophage populations is also considered to be a major driver in the pathogenesis of liver fibrosis but also during its regression [61]. So-called restorative macrophages (Ly-6C low) express MMPs and contribute to fibrolysis due to their phagocytic activity and regenerative capacities [62]. Inhibition of the monocyte chemoattractant protein 1 (C-C motif chemokine ligand 2) by the Spiegelmer-based pharmacologic inhibitor mNOX-E36 reduced the influx of infiltrating monocytes to the damaged liver, fostered the Ly-6C low phenotype and accelerated fibrosis resolution in two rodent models [43].

These data indicate that also cell-specific targeting of, for example, HSCs, myofibroblasts or macrophage populations can be potential targets for drug development in liver fibrosis, although the interplay of these cell populations still needs to be further investigated. Combination therapies targeting various cell types and pathways should therefore also be further explored preclinically. For a detailed overview on the numerous mediators identified to modulate inflammation and HSC activity, please see [21,30,61].

Various pathogenetic agents and conditions can lead to hepatocyte damage and activation of these inflammatory responses. While in chronic viral hepatitis (HBV and hepatitis C virus [HCV]), immune-cell-mediated destruction of hepatocytes is considered a main driver for maintaining inflammation, recent data also suggest a direct profibrogenic effect of HCV core and nonstructural proteins on HSCs [63].

Several pathways have been described to activate fibrotic responses in alcoholic liver diseases. Ethanol is mainly metabolized to acetaldehyde and then to acetic acid by a variety of cytosolic and mitochondrial enzymes (alcohol dehydrogenase and aldehyde dehydrogenase 2) in hepatocytes [64]. Acetaldehyde is considered to be the toxic metabolite that can directly activate collagen synthesis in HSCs but also interact with TGFβ receptors and other cytokines [65]. Moreover, ethanol can also be oxidized via CYP in the inducible microsomal ethanol oxidizing system when results in the generation of reactive oxygen species (ROS) that could directly damage hepatocyte. Further ethanol-related perturbations are lipid peroxidation and depletion of radical scavenger systems like vitamin C, E or glutathione, all leading to increased cellular stress conditions (including unfolded protein response and endoplasmic reticulum stress) that could trigger apoptotic cell death of hepatocytes. In addition, chronic ethanol consumption also increases the permeability of the gut wall to lipopolysaccharides, which activates TLR responses [66].

Progression from NAFLD to NASH is commonly associated with fibrosis and has also been linked to the release of proinflammatory cytokines from adipose tissues, ROS production, impaired lipid and cholesterol metabolism and intestinal lipopolysaccharides that penetrate the leaky gut wall with stimulation ofTLRs on Kupffer cells and HSCs [67-69]. Interestingly, also dietary fructose has been shown to promote fibrosis development by contributing to insulin resistance, obesity and steatosis [70,71]. It is thought that fructose induces lipogenesis in the liver and activates other inflammatory pathways, too. The imbalanced lipid metabolism, especially high levels of proinflammatory polyunsaturated fatty acids like linoleic acid or arachidonic acid further contribute to hepatic steatosis and inflammation [72]. The increased de novo lipogenesis induced by fructose depletes hepatocyte ATP levels leading to mitochondrial damage. Together with lipoxide and ROS production, this can also trigger cell death via endoplasmic reticulum stress-mediated activation of JNK, NFκB and autophagy pathways [73-76].

Recently, autophagy has also been shown to influence metabolic and inflammatory conditions linked to several pathophysiologic conditions in liver diseases [77]. Autophagy is a conserved mechanism by which damaged intracellular structures are subjected to lysosomal degradation and is considered to be a cellular survival mechanism as well as a backup energy production pathway under starvation conditions [78,79]. In brief, damaged organelles are sequestered into double-membrane autophagosomes under the control of various mediators like members of the ATG gene family, Beclin-1 or LC3, which themselves are controlled by key survival pathways like the PI3K/AKT/mTOR axis. The autophagosome is then fused with lysosomes to form the autophagolysosome, which leads to hydrolytic cleavage of its contents. For detailed review on the molecular mechanisms of autophagy and its physiologic and pathophysiologic roles in the liver, please refer to [79] and [80]. The hepatocyte-specific knockout of autophagy protein 5 (Atg5), an E3 ubiquitin ligase necessary for formation of the autophagosome, increased apoptosis of hepatocytes with inflammation and fibrosis, probably due to accumulation of polyubiquitinated proteins that could not be subjected to autophagy clearance. Interestingly, deletion of the inflammation-associated transcription factor Nrf2 in Atg5-deficient hepatocytes abrogated this phenotype [81].
Chronic viral hepatitis, both HBV and HCV, has been shown to involve autophagy processes to maintain viral replication [82-89], thus fostering inflammation and HSC activation, too. A key morphologic feature of activated HSCs is the loss of intracellular lipid storage, which has been demonstrated to be accompanied by an increased autophagy flux indicating that autophagy provides the energy needed for HSC activation [90-92]. Disturbances in lipid metabolism are also a key finding in NASH and metabolic syndrome-related liver fibrosis. Here, high insulin levels can activate the mTOR signaling cascade, which triggers autophagy and further influences mitochondrial dysfunction, fatty acid metabolism (both fostering ROS generation) and ER stress pathways [93-95]. Autophagy is also associated with proinflammatory cytokines in adipocytes [96]. Interestingly, autophagy has also been demonstrated to alleviate cellular stress conditions, indicating a context- and cell-type-dependent role of this pathway that could either induce or prevent hepatic fibrogenesis [97-100]. While the autophagy enhancer carbamazepine has been shown to diminish hepatocyte damage and reduce profibrogenic conditions in fibrinogen storage disease and in patients with α1-antitrypsin deficiency [101,102], inhibition of autophagy in HSCs by chloroquine also demonstrated to reduce fibrosis development in a CCl4 model [103], corroborating the context dependency of this mechanism.

3. Preclinical development options for antifibrotic agents

Today, treatment of liver fibrosis and cirrhosis is aiming at reducing clinical complications by, for example, reducing ascites or controlling bleeding of esophageal varices. A causal treatment is currently attempted by treating the underlying causative etiology, for example, by virus eradication, alcohol absence or control of metabolic dysfunctions [104]. Successful causative treatment can, in contrast to the longstanding textbook knowledge, lead to reversion of fibrosis and cirrhosis, which is supported by experimental and increasingly also clinical data [105,106]. Yet, despite the extensive knowledge on potential direct mechanistic targets for an antifibrotic therapy, clinical success for such treatment options has so far not been obtained. Among these targeted approaches, activation of fibrolysis by, for example, modulation of activity of matrix-degrading enzymes like MMPs or their inhibitors (TIMPs) as well as inhibition of matrix deposition by, for example, direct interference with HSC survival and function have been shown promising preclinical results in some models but disappointed in others and in clinical trials in humans [2,107-110].

As outlined above, the interplay between environmental and host factors is crucial for the development of fibrotic liver disease upon chronic inflammatory damage to the liver. Therefore, preclinical models are often not sufficiently close to the human pathophysiologic conditions by, for example, lacking the immunologic interface or applying pathophysiologic conditions that are not representative for the human situation (e.g., application of thioacetamide of concavalin A). Bile duct ligation models might be representative for conditions of biliary cirrhosis but do not reflect changes observed in, for example, NASH or viral hepatitis [107,108]. To overcome these obstacles, more advanced preclinical models are needed and have, in part, already been established, for example, the Mdr2 (Abcb4−/−) knockout mouse [111] or the ApoE-LDLR double knockout mouse [112]. Although these approaches are highly relevant to understand distinct pathophysiologic conditions, they might not be representative for the majority of human disease conditions leading to chronic liver injury and fibrosis development as these are usually not monogenetic diseases [15,107]. Furthermore, humanized mouse models represent an attractive and easily accessible alternative, especially for chronic viral hepatitis models [113,114]. Recently, also mouse models reflecting the human immune system were established that provide an excellent approach for drug development studies in rodents [115-117].

Despite the above-mentioned limitations of the animal models, several of these are used for testing of new antifibrotic liver agents. For the determination of treatment effects the investigators often use two of the most important read-out parameters like those given in human clinical studies, the NAFLD activity score (NAS) and fibrosis stage [118] and increasingly the portal vein pressure measurement, which correlated significantly with the stage of cirrhosis in the study of Traber et al. [119].

In some labs, also larger animals are used for translation of antifibrotic drugs into the clinics. For example, a cynomolgus monkey model using CCl4 injections was established, which closely resembles human disease conditions [120] and also allows serial biopsies of liver tissue and to monitor disease progression more closely, and is thus providing a clear advantage over small animal models [121]. The CCl4 model was also successfully transferred to minipigs [122] and is used here for various MRI-based imaging approaches for staging of fibrosis [123-125]. In minipigs, also a dietary model for steatohepatitis has recently been described [126] and various models for alcoholic liver damage exist.

A further interesting approach represents a relatively new in vitro technique, the so-called precision-cut liver slices (250 μm) to study fibrosis mechanisms and potential interventional targets. This model system is clearly superior to conventional cell-culture systems as it maintains the tissue architecture, contains several key cell types (e.g., hepatocytes, biliary tract cells and HSCs) and still reflects immunologic mechanisms from the host organism to various pathogenetic stimuli, including CCl4, bile acids, thioacetamide or acetaminophen [127-130]. Liver slices have recently been used to evaluate the effect of antifibrotic drugs and it was shown that various agents (e.g., TGFβ pathway inhibitors like perindopril or PDGF inhibitors like sorafenib) demonstrate antifibrotic effects here, too [131-133], and could potentially be used as a screening tool for future drug development activities.
4. Clinical development options for antifibrotic agents

Human translational studies are often hampered by the complex pathophysiology with various underlying causative agents (e.g., toxic, infectious) and subtypes within these groups (e.g., HBV vs HCV, viral genotypes, host factors) that often lead to only small numbers of cases with limited statistical power, especially when genetic analyses are performed. Rapid and successful clinical development of antifibrotic agents is limited by the long interval between initiation and progression of the disease until full clinical symptoms have developed as well as by the lack of validated biomarkers and clear clinical endpoints accepted by regulatory authorities [107,108,110].

Liver biopsy remains the gold standard for assessing fibrotic changes as standard serum parameters like transaminases or albumin or quantitative testings of liver function are usually altered either unspecifically or at advanced disease stages only. Yet, liver biopsy represents an invasive procedure with a potential risk to patients (especially with advanced and decompensated fibrosis) and shows significant sampling error as well as interobserver variability [134-136]. Therefore, noninvasive methods for assessing fibrosis have entered the focus of translational research. Conventional imaging techniques, including MRI and sonography, failed to differentiate fibrosis stages but image guides measurement of hepatic stiffness (transient elastography, acoustic radiation force imaging, supersonic shear wave imaging or MR elastography) has demonstrated good accuracy [136,137]. Using collagen I-specific MRI probes (EP-3533), a sensitive noninvasive imaging biomarker was established in mice and rats that could help generate early signals in clinical studies [138,139]. Although not yet clinically validated, this approach demonstrated a good diagnostic specificity and sensitivity to distinguish early (Ishak Grade 3) from late (Ishak Grade 4) fibrosis and was superior to other imaging approaches. A plethora of either direct (reflecting ECM metabolism) or indirect (reflecting liver function) serum marker panels have been proposed and investigated in numerous studies [140,141]. Indirect markers like AST-platelet ratio index, Fibrotest, FIB4, NAFLD Fibrosis score or Fibroindex combine various biochemical and clinical parameters and are usually inexpensive and easily accessible. These tests yield good values in AUROC analysis but are often only validated in distinct disease conditions. Direct markers monitor levels of, for example, hyaluronic acid, procollagen III peptide or TIMP-1. Here, several neo-epitopes from MMP-mediated cleavage of collagen peptides have recently been identified [142-144]. First clinical data from patients with HCV indicate that one of these markers, the N-terminal type III collagen propeptide (Pro-C3), can distinguish early (Ishak Grade 2/3) from advanced (Ishak Grade 4) fibrosis from plasma samples with superiority to Fibrotest, which clearly warrants further investigations [145]. The Enhanced Liver Fibrosis (ELF) score combines these parameters with age and reported good sensitivity and AUROC values for NAFLD and viral hepatitis [146,147]. Combinations of serum parameters and imaging techniques could further increase their predictive value and have already been predicted as end points for clinical trials [148,149]. Recently, the hepatic vein pressure gradient (HVPG) has been established as a predictive marker in cirrhosis and was shown to correlate with hepatic collagen content [150-152].

Careful selection of patient populations and adequate endpoints is crucial for successful and innovative clinical trials and to obtain regulatory approval for antifibrotic therapies. It was suggested that patients with intermediate stage of fibrosis and faster dynamics in disease progression, for example, posttransplant HCV patients who have a risk of accelerated fibrosis progression of 30% in 3–5 years, should be included in clinical trials to have an optimal readout of the study in a reasonable time frame [15,107,108,153]. Especially in these patients HVPG could be an appropriate study endpoint [107]. Several genetic polymorphisms have been identified as potential risk factors for fibrosis development and progression under various etiologic conditions, for example, rs12104272 in HCV [154], or variants of CYP7A1 in biliary cirrhosis [155]. A missense SNP of the ATP-dependent RNA helicase DDX5 was recently identified in HSCs and is associated with enhanced expression of profibrogenic genes [156]. These results show that genetic polymorphisms can be used to identify risk populations with various etiologic backgrounds but also independent of the underlying pathophysiology and could therefore also be used for selecting patients with high risk of fibrosis progression.

A recent joint workshop from FDA and AASLD on trial designs and endpoints for liver disease secondary to NAFLD addressed the questions and also recommended to include patients with the highest risk into trials. Although improved survival was identified as the most meaningful endpoint, surrogate endpoints in regard of the longlasting course of the disease could also be regression of fibrosis, prevention of fibrosis progression and improved quality of life [157]. Furthermore, disease-specific endpoints for clinical studies have been proposed for, for example, viral hepatitis, NASH [158] and biliary cirrhosis [159] by AASLD and other organizations [160]. For NASH, it is recommended to use the NAS for disease activity staging and to use liver biopsies for staging in Phase II and Phase III trials. Subjects being at risk for developing cirrhosis should be included into NASH studies at highest priority and primary endpoints were proposed to be resolution of steatohepatitis, improvement in NAS as well as MR spectroscopy and improvement in biochemical parameters, especially for short-duration Phase I trials where sequential liver biopsy is not feasible. For cirrhotic patients with NASH, changes in Child-Pugh and model for end-stage liver disease (MELD) scores as well as signs of clinical decompensation are suggested as primary study endpoints. Overall, selection of patients with established cirrhosis needs to be done carefully. Although endpoints like death, transplantation or development of HCC are clinically meaningful, they are considered not to be feasible
due to long follow-up times and large cohort sizes. We also refer the reader to the results of the recently held AASLD workshop on “Emerging Trends in Antifibrotic Drug Trials: Strategies and Endpoints,” which will provide an in-depth coverage of regulatory questions and endpoints for clinical trials in this setting.

5. Current clinical trials

Several Phase I and II trials have been identified that investigate the antifibrotic properties of various agents (Table 1). Trials that directly target distinct underlying etiologies like HBV or HCV replication are not listed here. Various cell-based approaches, using bone marrow– or umbilical cord–derived mesenchymal progenitor or stem cells as well as cells derived from peripheral blood, are currently under investigation (Table 2). Experimental data indicate that these cell types can repopulate to the liver and differentiate into mature hepatocytes to compensate for the parenchymal damage induced by the different underlying pathophysiological conditions. Furthermore, cell transplantations have also been shown to exert immunomodulatory effects by interacting with various immune cells and thus have a dual effect on both parenchymal damage and inflammatory conditions. Umbilical cord or bone marrow–derived mesenchymal stem cells are currently used in various Phase I and II trials. Pilot studies have already demonstrated good tolerability in biliary [161] or alcoholic cirrhosis [162] and showed early signs of activity here, while a randomized trial in patients with decompensated cirrhosis did not show any benefit [163] so that further studies are well justified and needed at this point in time. Pharmacologic approaches to target stem-cell differentiation are investigating the use of the smoothened inhibitor LDE225 or the CBP/catenin antagonist PRI-724.

As the focus of this article is on targeted agents, we refer to [164] for further details on cell-based therapies. Investigational agents in Phase I and II clinical trials show a broad range from vitamins to targeted agents and monoclonal antibodies (Table 1). Interestingly, also the applied primary and secondary endpoints show a huge variety within these trials, ranging, for example, from overall survival to morphometric quantification of collagen content. Other trials use surrogate endpoints related to the underlying etiology, for example, pruritus in PBC or ascites volume in nonspecified cirrhotic conditions. Several trials apply biopsy-based endpoints like liver histology at various time points, while others use noninvasive measures like Fibroscan. Only one trial was found investigating HVPG as a primary endpoint together with the event-free survival of patients with NASH. Secondary endpoints are even less well defined, applying also noncirrhosis scores like MELD or Child-Pugh. Quality of Life was rarely addressed. Further consolidation of clinically feasible and regulatory acceptable endpoints is still urgently needed to have successful trials and comparable results for clinical development and efficacy assessment.

Serelaxin (RLX030) is a recombinant human relaxin-2 that binds to a G-protein–coupled receptor family (RXFP) and has been demonstrated to influence cardiac remodeling in humans and other fibrogenic processes in animal models [165]. Data from liver fibrosis are pending.

The pan-caspase inhibitor IDN-6556 (Emricasan) has been granted orphan drug status by the FDA for the treatment of liver diseases. Preclinically, IDN-6556 attenuated liver fibrosis in murine bile duct ligation and steatohepatitis models [166,167]. Oral treatment with IDN-6556 in patients with HCV or NASH reduced transaminase levels in a first clinical study [168].

The human monoclonal anti-CTGF antibody FG-3019 was investigated in various animal models of fibrotic diseases and demonstrated a reduction on direct fibrosis markers like histology or hydroxyproline content but still lacks proof of concept in liver fibrosis settings [169].

Anthocyanins are plant-derived antioxidants and are commonly used in traditional medicine. They decrease oxidative stress and lipid peroxidation and have been shown to improve cholestatic liver damage in a bile duct ligation mouse model [170]. Additionally, proliferation of HSCs was inhibited in rats [171,172], indicating a potential antifibrotic effect in various pathophysiologic settings, including NASH [173]. Other antioxidants like vitamin E can also contribute to improve hepatic inflammation and fibrotic remodeling [174].

Galecins are cell-surface glycoproteins that can mediate cell migration, matrix interaction and inflammatory signals. Galectin inhibitors like GR-MD-02 showed promising results in a thioacetamide and in a NASH rat model of liver fibrosis leading to reduced histology staging and even dissolution of cirrhosis, accompanied by reduced inflammation and portal pressure [118,119].

Other compounds in current clinical trials aim to target clinical symptoms rather than ameliorating the fibrotic change itself. Among them, sildenafil is used to reduce HVPG, although a first trial could not demonstrate a clinically relevant influence here [175]. Various studies also investigate the effect of hypocaloric diet and exercise, insulin sensitizer like sitagliptin or pioglitazone, lipid-lowering drugs like atorvastatin or ezetimibe and other nutritional antioxidants like vitamin A or D [176-179].

Although a strong preclinical rationale is usually given, first clinical trials could not always show clear results thus requiring additional and larger trials with better stratified patient populations and novel endpoints. Interestingly, the farnesoid X receptor agonist INT-747 (obeticholic acid), a derivative of a primary human bile acid, has successfully finished two randomized, double-blind Phase II studies in PBC and NAFLD [180,181]. Besides ameliorating surrogate parameters of liver damage (e.g., alkaline phosphatase levels) over several months, INT-747 was recently shown to exhibit beneficial additional effects on portal hypertension in a rat model [182]. These data indicate that also rather unspecific biochemical surrogate parameters of liver damage can lead to successful
Table 1. Current clinical trials with potential antifibrotic agents.

<table>
<thead>
<tr>
<th>NCT no.</th>
<th>Agents</th>
<th>Disease</th>
<th>Phase</th>
<th>Design</th>
<th>Primary endpoints</th>
<th>Secondary endpoints</th>
<th>Molecular target/mechanism</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02029586</td>
<td>β-lapachone (MB12066)</td>
<td>NAFLD</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>NAFLD activity score (steatosis, 12 weeks)</td>
<td>Histology, fibrosis/steatosis' inflammation</td>
<td>Vasodilatation via activation of NAD(P)H:quinone oxidoreductase 1 (NQO1) [186]</td>
<td>KT&amp;G Life Sciences Corp</td>
</tr>
<tr>
<td>NCT01940263</td>
<td>Anthocyanin</td>
<td>NAFLD</td>
<td>0</td>
<td>Randomized, double blind</td>
<td>Oxidative stress</td>
<td>Inflammation</td>
<td>Antioxidant [187]</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01617772</td>
<td>Atorvastatin, L-carnitine</td>
<td>NASH</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>Fibroscan (2 years)</td>
<td>Liver enzymes, safety</td>
<td>HMG-CoA reductase inhibitors lower cholesterol and (atorvastatin) binds fatty acids (L-carnitine)</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01379469</td>
<td>Carbamazepine</td>
<td>A1ATD</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>Hepatic ATZ load</td>
<td>Fibrosis histology, MELD (12 months)</td>
<td>Decreases mutant ATZ by increasing autophagy and proteasomal degradation [101]</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT02077374</td>
<td>Emricasan (IDN-6556)</td>
<td>NAFLD</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>Alanine amino-transferase (28 days) PK</td>
<td>AST, CK18, caspases, insulin</td>
<td>Pan-caspase inhibitor [167]</td>
<td>Conatus Pharmaceuticals, Inc.</td>
</tr>
<tr>
<td>NCT02138253</td>
<td>Emricasan (IDN-6556)</td>
<td>HCV</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>Ishak score (2 years)</td>
<td>Necro-inflammatory subscore of the modified Histological Activity Index</td>
<td>Pan-caspase inhibitor [167]</td>
<td>Conatus Pharmaceuticals, Inc.</td>
</tr>
<tr>
<td>NCT02151864</td>
<td>Erismodegib (LDE225)</td>
<td>HCC</td>
<td>I</td>
<td>Single arm, open label</td>
<td>Safety</td>
<td>Safety</td>
<td>Smoothened (Smo)/Sonic hedgehog (Shh) pathway inhibitor</td>
<td>Academic, Novartis</td>
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<tr>
<td>NCT01766713</td>
<td>Ezetimibe</td>
<td>NASH</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>Liver biopsy (24 weeks)</td>
<td>Safety</td>
<td>Decreases cholesterol absorption in small intestine [188]</td>
<td>Academic</td>
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<tr>
<td>NCT02193360</td>
<td>FFP104</td>
<td>PBC</td>
<td>III</td>
<td>Single arm, open label</td>
<td>Safety</td>
<td>Biochemistry, pruritus, QoL</td>
<td>Monoclonal anti-CD40 antibody, immunomodulatory effects [189]</td>
<td>Fast Forward Pharmaceuticals</td>
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<td>NCT01217632</td>
<td>FG-3019</td>
<td>HBV</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>Efficacy in liver fibrosis</td>
<td>Safety</td>
<td>Anti-CTGF monoclonal antibody [190]</td>
<td>FibroGen</td>
</tr>
</tbody>
</table>

Source: clinicaltrials.gov.

A1ATD: α1-Antitrypsin deficiency; AE: Adverse events; AFP: α-Fetoprotein; ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase; ATZ: Mutant α1-antitrypsin Z; CK18: Cytokeratin 18; CP: Child-Pugh score; ECG: Electrocardiogram; gGlutamyl transferase; HBV: Hepatitis B virus; HCV: Hepatitis C virus; MELD: Model for end-stage liver disease; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; OS: Overall survival; PBC: Primary biliary cirrhosis; PD: Pharmacodynamics; PK: Pharmacokinetics; PSC: Primary sclerosing cholangitis; QoL: Quality of Life; RBC: Red blood cell count.
<table>
<thead>
<tr>
<th>NCT no.</th>
<th>Agents</th>
<th>Disease</th>
<th>Phase</th>
<th>Design</th>
<th>Primary endpoints</th>
<th>Secondary endpoints</th>
<th>Molecular target/ mechanism</th>
<th>Sponsor</th>
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<tr>
<td>NCT01056133</td>
<td>Fish oil</td>
<td>NASH</td>
<td>II</td>
<td>Single arm, open label</td>
<td>Liver histology (12 months)</td>
<td>Plasma and RBC fatty acid composition, biochemistry, intestinal microbiota, endotoxin, free cholin, bacterial DNA MR biomarkers</td>
<td>Change of hepatic lipid content (polyunsaturated fatty acids) [191]</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01930123</td>
<td>Fructose</td>
<td>NAFLD</td>
<td>I</td>
<td>Single arm, open label</td>
<td>Metabolism and energy homeostasis</td>
<td>PK, ALT, AST, gGT, ALP, PD biomarkers</td>
<td>Effect on metabolic syndrome and glucose homeostasis [192]</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01899859</td>
<td>GR-MD-02</td>
<td>NASH</td>
<td>I</td>
<td>Randomized, double blind</td>
<td>Safety</td>
<td>Liver fat content, liver functions, inflammation</td>
<td>Galectin inhibitor [118,119]</td>
<td>Galectin Therapeutics, Inc.</td>
</tr>
<tr>
<td>NCT01477307</td>
<td>Hypocaloric diet</td>
<td>NAFLD</td>
<td>II</td>
<td>Observational</td>
<td>Fecal bacterial cells</td>
<td>Liver fat MRI and spectroscopy, liver function tests, lipids, glucose, body weight</td>
<td>Effort on metabolic syndrome and glucose homeostasis [192]</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01679197</td>
<td>Leptin</td>
<td>NASH/ NAFLD</td>
<td>II</td>
<td>Single arm, open label</td>
<td>Liver histology (12 months)</td>
<td>Unknown, cytokine signaling in adipose tissue</td>
<td>Unknown, cytokine signaling in adipose tissue</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01904058</td>
<td>LUM001</td>
<td>PBC</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>Efficacy (13 weeks, pruritus)</td>
<td>Liver enzymes, safety</td>
<td>Inhibits apical sodium-dependent bile acid transporter (ASBT), reduces bile acid absorption</td>
<td>Lumena Pharmaceuticals, Inc.</td>
</tr>
<tr>
<td>NCT02173288</td>
<td>Midodrine and</td>
<td>Cirrhosis</td>
<td>II/III</td>
<td>Randomized, open label</td>
<td>Control of ascites (3 months)</td>
<td>Encephalopathy, liver function, variceal bleeding, hepatorenal syndrome, hypernatremia Bilirubin</td>
<td>Vasoconstrictor (midodrine); vasopressin receptor antagonist (tolvaptan)</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT02026401</td>
<td>NGM282</td>
<td>PBC</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>ALP</td>
<td>FGF19 analog, influence bile acid transport and glucose metabolism [194]</td>
<td>NGM Biopharmaceuticals, Inc.</td>
<td></td>
</tr>
<tr>
<td>NCT00570765</td>
<td>Obeticholic acid</td>
<td>PBC</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>ALP</td>
<td>gGT, ALT, PK</td>
<td>NGM Biopharmaceuticals, Inc.</td>
<td></td>
</tr>
</tbody>
</table>

Source: clinicaltrials.gov.

A1ATD: α1-Antitrypsin deficiency; AE: Adverse events; AFP: α-Fetoprotein; ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase; ATZ: Mutant α-1-antitrypsin Z; CK18: Cytokeratin 18; CP: Child-Pugh score; ECG: Electrocardiogram; gGT: γ-Glutamyl transferase; HBV: Hepatitis B virus; HCV: Hepatitis C virus; MELD: Model for end-stage liver disease; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; OS: Overall survival; PBC: Primary biliary cirrhosis; PD: Pharmacodynamics; PK: Pharmacokinetics; PSC: Primary sclerosing cholangitis; QoL: Quality of Life; RBC: Red blood cell count.
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<thead>
<tr>
<th>NCT no.</th>
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<th>Phase</th>
<th>Design</th>
<th>Primary endpoints</th>
<th>Secondary endpoints</th>
<th>Molecular target/mechanism</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00550862</td>
<td>Obeticholic acid (INT-747) + ursodeoxycholic acid</td>
<td>PBC</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>ALP</td>
<td>gGT, ALT, PK</td>
<td>Farnesoid X receptor agonist, regulates lipids, glucose and bile acids [182]</td>
<td>Intercept Pharmaceuticals</td>
</tr>
<tr>
<td>NCT01434108</td>
<td>Ornithine Phenylacetate</td>
<td>Cirrhosis</td>
<td>I/VIII</td>
<td>Randomized, double blind</td>
<td>Ammonia plasma concentration</td>
<td>Hepatic encephalopathy</td>
<td>Substrate for glutamine synthase (L-ornithine) and increase excretion of glutamine (phenylacetate) [195]</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01068444</td>
<td>Pioglitazone</td>
<td>NASH</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>Steatosis and liver function tests (9 months), safety</td>
<td>Liver histology</td>
<td>Antidiabetic, insulin sensitizer [196]</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT02195440</td>
<td>PRI-724</td>
<td>HCV</td>
<td>I</td>
<td>Single arm, open label</td>
<td>CP, liver biopsy, albumin, serum fibrosis markers, ascites, edema, PK</td>
<td>Inflammation, liver enzymes, metabolic parameters, immunology and viral load</td>
<td>β-catenin antagonist [197]</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01231685</td>
<td>Raltegravir</td>
<td>HIV-HCV</td>
<td>II</td>
<td>Randomized, open label</td>
<td>Fibroscan, AST-platelet ratio index, 48 weeks</td>
<td>Hospitalization rate, PK, AE, lab values, ECG, QoL</td>
<td>Inhibitor or retrovirus integrase</td>
<td>Academic, MSD</td>
</tr>
<tr>
<td>NCT01904409</td>
<td>Rifaximin</td>
<td>Cirrhosis</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>Time to mortality or hospitalization</td>
<td>Safety</td>
<td>Poorly absorbed antibiotic; inhibits bacterial RNA synthesis in the gut, which reduces microbial production of neurotoxic metabolites [198]</td>
<td>Salix Pharmaceuticals</td>
</tr>
<tr>
<td>NCT01703260</td>
<td>Roflumilast and Pioglitazone</td>
<td>NASH</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>ALT (4 months)</td>
<td>AST, liver fat content</td>
<td>Phosphodiesterase-4 inhibitor, anti-inflammatory</td>
<td>Takeda</td>
</tr>
<tr>
<td>NCT01640964</td>
<td>Serelaxin (RLX030)</td>
<td>Alcohol</td>
<td>II</td>
<td>Randomized, open label</td>
<td>Total renal artery flow</td>
<td>Hemodynamic measurements, portal vein pressure</td>
<td>Recombinant human relaxin-2 peptide; vasodilatation via RXFP1 and RXFP2 receptor binding [165]</td>
<td>Novartis</td>
</tr>
<tr>
<td>NCT01954524</td>
<td>Sildenafil</td>
<td>Cirrhosis</td>
<td>I</td>
<td>Single arm, open label</td>
<td>Safety</td>
<td></td>
<td>Inhibitor of phosphodiesterase-5, vasodilator</td>
<td>Academic</td>
</tr>
</tbody>
</table>

Source: clinicaltrials.gov.
A1ATD: α1-Antitrypsin deficiency; AE: Adverse events; AFP: α-Fetoprotein; ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase; ATZ: Mutant α1-antitrypsin Z; CK18: Cytokeratin 18; CP: Child-Pugh score; ECG: Electrocardiogram; gGT: γ-Glutamyl transferase; HBV: Hepatitis B virus; HCV: Hepatitis C virus; MELD: Model for end-stage liver disease; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; OS: Overall survival; PBC: Primary biliary cirrhosis; PD: Pharmacodynamics; PK: Pharmacokinetics; PSC: Primary sclerosing cholangitis; QoL: Quality of Life; RBC: Red blood cell count.
Table 1. Current clinical trials with potential antifibrotic agents (continued).

<table>
<thead>
<tr>
<th>NCT no.</th>
<th>Agents</th>
<th>Disease</th>
<th>Phase</th>
<th>Design</th>
<th>Primary endpoints</th>
<th>Secondary endpoints</th>
<th>Molecular target/mechanism</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT01672866</td>
<td>Simtuzumab</td>
<td>NASH</td>
<td>IIb</td>
<td>Randomized, double blind</td>
<td>Quantitative morphometry of collagen content, 96 weeks</td>
<td>Safety</td>
<td>Humanized monoclonal anti-lysyl oxidase homolog 2 (LOXL2) antibody, immunomodulator [50]</td>
<td>Gilead</td>
</tr>
<tr>
<td>NCT01672879</td>
<td>Simtuzumab</td>
<td>NASH</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>Hepatic venous pressure gradient (96 weeks), event-free survival time</td>
<td>Safety</td>
<td>Humanized monoclonal anti-lysyl oxidase homolog 2 (LOXL2) antibody, immunomodulator [50]</td>
<td>Gilead</td>
</tr>
<tr>
<td>NCT01672853</td>
<td>Simtuzumab</td>
<td>PSC</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>Quantitative morphometry of collagen content, 96 weeks</td>
<td>Safety</td>
<td></td>
<td>Gilead</td>
</tr>
<tr>
<td>NCT01963845</td>
<td>Sitagliptin</td>
<td>NAFLD</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>Steatosis (MRI scan, 24 weeks)</td>
<td>Liver histology,</td>
<td>Antidiabetic agent, inhibits dipeptidyl peptidase 4 [188]</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01919294</td>
<td>Testosterone</td>
<td>NASH</td>
<td>II</td>
<td>Single arm, open label</td>
<td>Steatosis histology (12 months)</td>
<td>Biochemistry, safety</td>
<td></td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01571063</td>
<td>Vitamin D</td>
<td>NASH</td>
<td>II</td>
<td>Randomized, single blind</td>
<td>Alanine aminotransferase (48 weeks)</td>
<td>Liver histology,</td>
<td>Substitution of low levels in NASH, immunomodulatory effects [199]</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01792115</td>
<td>Vitamin E</td>
<td>NAFLD</td>
<td>II</td>
<td>Randomized, open label</td>
<td>Transaminases (9 months), liver fat content</td>
<td>Gene expression,</td>
<td>Antioxidant</td>
<td>Academic</td>
</tr>
</tbody>
</table>
<pre><code>                                                                                                                                      | oxidative stress, NH/NKT cell phenotype                        |                     |                                                                                |          |
</code></pre>

Source: clinicaltrials.gov.

A1ATD: α1-Antitrypsin deficiency; AE: Adverse events; AFP: α-Fetoprotein; ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase; ATZ: Mutant α1-antitrypsin Z; CK18: Cytokeratin 18; CP: Child-Pugh score; ECG: Electrocardiogram; gGT: γ-Glutamyl transferase; HBV: Hepatitis B virus; HCV: Hepatitis C virus; MELD: Model for end-stage liver disease; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; OS: Overall survival; PBC: Primary biliary cirrhosis; PD: Pharmacodynamics; PK: Pharmacokinetics; PSC: Primary sclerosing cholangitis; QoL: Quality of Life; RBC: Red blood cell count.
Table 2. Current clinical trials with cell-based approaches.

<table>
<thead>
<tr>
<th>NCT no.</th>
<th>Agents</th>
<th>Disease</th>
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<th>Design</th>
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<th>Secondary endpoints</th>
<th>Sponsor</th>
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<td>NCT01591200</td>
<td>Mesenchymal stem cells</td>
<td>Alcohol</td>
<td>II</td>
<td>Randomized, open label</td>
<td>Safety</td>
<td>Liver function, CT scans, MELD, QoL, CP, liver biopsy</td>
<td>Stempeutics Research Pvt Ltd</td>
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<tr>
<td>NCT01877759</td>
<td>Mesenchymal stem cells</td>
<td>Cirrhosis</td>
<td>I/II</td>
<td>Single arm, open label</td>
<td>Liver function tests 1-year survival</td>
<td>MELD, CP, QoL, MELD, CP, AFP, renal function</td>
<td>Academic</td>
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<tr>
<td>NCT01728727</td>
<td>Mesenchymal stem cells</td>
<td>HBV</td>
<td>I/II</td>
<td>Randomized, open label</td>
<td>CP, MELD</td>
<td>Fibroscan, QoL, liver function</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT02171949</td>
<td>Mononuclear bone marrow cells</td>
<td>Cirrhosis</td>
<td>II</td>
<td>Randomized, double blind</td>
<td>CP, MELD</td>
<td>Fibroscan, QoL, liver function</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01728688</td>
<td>Autologous peripheral blood stem cells</td>
<td>HBV</td>
<td>I/II</td>
<td>Randomized, open label</td>
<td>1-year survival</td>
<td>MELD, CP, AFP, renal function</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01741090</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
<td>Alcohol</td>
<td>II</td>
<td>Single arm, open label</td>
<td>Liver histology (6 months)</td>
<td>Immunohistochemistry, direct fibrosis parameters, hepatic venous pressure gradient, hepatic vein arrival time, liver stiffness, CP, MELD</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01875081</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
<td>Alcohol</td>
<td>II</td>
<td>Randomized, open label</td>
<td>Fibrosis grade (histologic, 6 months)</td>
<td>Histology, MELD, CP, liver function tests, Fibroscan, liver volume</td>
<td>Pharmicell Co., Ltd.</td>
</tr>
<tr>
<td>NCT01724697</td>
<td>Bone marrow-derived mesenchymal stem cells</td>
<td>HBV</td>
<td>I/II</td>
<td>Randomized, open label</td>
<td>1-year survival</td>
<td>MELD, CP, AFP, renal function</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01491165</td>
<td>Umbilical cord mesenchymal stem cells</td>
<td>Cirrhosis</td>
<td>I/II</td>
<td>Single arm, open label</td>
<td>Liver volume (MRI scan)</td>
<td>Liver biopsy, gastroscopy, biochemistry, portal and splenic vein measurements</td>
<td>Academic</td>
</tr>
<tr>
<td>NCT01662973</td>
<td>Umbilical cord mesenchymal stem cells</td>
<td>PBC</td>
<td>I/II</td>
<td>Randomized, open label</td>
<td>ALP</td>
<td>Liver biopsy, bilirubin, AST, Mayo risk score, portal hypertension, MELD, clinical</td>
<td>Academic</td>
</tr>
</tbody>
</table>

Source: clinicaltrials.gov.

AE: Adverse events; AFP: α-Fetoprotein; ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase; CP: Child-Pugh score; HBV: Hepatitis B virus; MELD: Model for end-stage liver disease; PBC: Primary biliary cirrhosis.
endpoints in clinical trials and should therefore be further evaluated and used for antifibrotic drug development.

6. Conclusion

Numerous approaches for the direct treatment of fibrotic liver disease are currently in early-phase clinical development. Besides cell-based approaches using stem and precursor cell populations to compensate for parenchymal damage, several targeted concepts are under investigation. Here, focusing on key inflammatory and signaling pathways seems to provide promising preclinical data, although these approaches still need validation in humans. Preclinical approaches using novel \textit{in vitro} systems like precision-cut liver slices and new \textit{in vivo} systems like genetically modified or humanized mouse models as well as potential large animal models can provide a better basis for effective treatment in humans. In clinical trials, careful selection of the patient population and the use of novel biomarker-based or imaging-based trial endpoints can contribute to establish successful therapies for fibrotic liver diseases in the near future.

7. Expert opinion

Chronic liver diseases still represent a high unmet medical need and are predicted to further increase in incidence in the next decades due to the high prevalence of chronic viral hepatitis and especially the steep increase on NAFLD and NASH worldwide. The common end-stage of these chronic inflammatory conditions is extensive parenchymal damage leading to dysregulated wound repair, tissue remodeling and cell proliferation, which is morphologically seen as liver fibrosis, regenerative nodules, shunt circulations and, at last, development of hepatic failure and hepatocellular carcinoma in the cirrhotic liver. So far, treatment of liver fibrosis and cirrhosis is symptomatic and related to the underlying pathophysiologic agents, for example, achieving sustained virologic response in chronic viral hepatitis B or C. Especially here, novel agents have led to a significant improvement for patients and are also expected to reduce the development of fibrotic and cirrhotic liver disease in the coming years. Yet, despite extensive knowledge about the pathophysiologic mechanisms leading to hepatocyte apoptosis, inflammation and finally activation of HSCs with a quantitatively and qualitatively altered deposition of ECM, little progress has been made in translating these findings into selective and potent targeted agents in humans so far. Idiopathic lung fibrosis can be seen here as a pivotal example that the successful development of antifibrotic agents can be achieved. While previous therapies focused on the use of anti-inflammatory and immunosuppressant agents like steroid or azathioprine, pirfenidone has been approved as a novel antifibrotic therapeutic agent here and provides clear clinical benefit to patients now [183,184].

The currently available animal models for liver fibrosis, for example, genetically engineered mice, bile duct ligation or toxic injury models like concavalin A, thioacetamide or CCl\textsubscript{4} injections, do usually not reflect the complex human pathophysiologic. While these models focus on a rather acute damage with distinct pathophysiologic, the human situation is characterized by various additional environmental factors like, for example, genotype, immunologic processes and nutritional status, and, importantly, a time course spanning up to several decades between initial injury and fully developed cirrhosis. Especially for diseases like NAFLD and NASH, dietary models and genetic models like the Mdr2 (Abcb4\textsuperscript{-/-}) knockout mouse or the ApoE-LDLDR double knockout mouse hold great promises for the successful translation of preclinical data to humans and additional effort should be put into using these models alone or in combination with other pathophysiologic principles. It is also important to emphasize that potential antifibrotic agents need to demonstrate efficacy in more than one model and in more than one animal species to better reflect the heterogeneity of the human pathophysiologic. In this context, the use of non-rodent models like cynomolgus monkeys or minipigs is strongly recommended, too. These species resemble the human situation more closely than small animals and allow to monitor the course of the disease by taking serial biopsies, blood samples and imaging approaches as in humans. It is also strongly recommended to use precision-cut liver slices extensively as a biologically relevant \textit{in vitro} screening tool to overcome limitations of small and large animal models in early phases of drug development. This system has been intensively validated but is still not used to the extent necessary to overcome the limitations current preclinical models have.

The clinical development of direct antifibrotic agents, for example, inhibitors of matrix deposition by HSCs, activation of fibrolytic pathways or modulation of the immune response, is still considered a major challenge. Liver biopsy represents the gold standard for clinical assessment of fibrosis, although recently noninvasive approaches using imaging techniques, serum parameters or sonography methods have become more reliable and are used more widely. Early clinical trials in drug development should provide a rapid and robust readout. Therefore, these techniques could also lead to the definition of novel endpoints in clinical trials and overcome current regulatory hurdles like the high inter- and intraobserver variability of liver biopsies. Surrogate parameters like HVPG or noninvasive measurement of liver stiffness as well as serum scores could thus enter the focus of clinical development. It is noteworthy that surrogate biomarkers can be accepted by regulatory authorities if they possess a reasonable likelihood of predicting clinical benefit for the patient and more effort needs to be taken to validate those readouts also during early clinical studies and even in preclinical settings. We also refer the reader to the respective FDA guidance on approval to market new drugs [185]. Also, the selection of patients is crucial to obtain positive study readout and patients with
intermediate-stage fibrosis should be preferred for clinical studies as the high dynamics in this population will allow for faster readout also of surrogate parameters. To overcome obstacles related to liver biopsy for the assessment of fibrosis and inflammation, surrogate parameters like HVPG, serum-based scores and imaging techniques can be applied and provide a reliable and rapid readout.

Various targeted agents are undergoing early-phase clinical programs. While a strong preclinical and experimental rationale is available for most of these assets, clinical data are still lacking and sometimes also controversial so that additional trials with better-defined patient populations and endpoints are urgently needed. Interestingly, most of these agents are inhibitors of profibrogenic pathways interfering with inflammatory response, matrix deposition or HSC activation. Currently, no agent is aiming at activating fibrolysis although this approach, if selective enough, could be a powerful tool to reverse tissue remodeling and penetrate the vicious circle of hepatocyte damage, persistent inflammation and matrix deposition.

With the emerging high prevalence of metabolic disorders leading to NAFLD and NASH, additional awareness on liver diseases and increased research activities is needed in the future.

**Declaration of interest**

All of the authors are employees of Bayer Pharma AG. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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** FDA guideline for clinical trial endpoints.


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