# Evolving therapies for liver fibrosis

Detlef Schuppan[1,2] and Yong Ook Kim[1]

[1]Institute of Molecular and Translational Medicine and Department of Medicine I, University Medical Center, Johannes Gutenberg University Mainz, Mainz, Germany. [2]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA.

**Fibrosis is an intrinsic response to chronic injury, maintaining organ integrity when extensive necrosis or apoptosis occurs. With protracted damage, fibrosis can progress toward excessive scarring and organ failure, as in liver cirrhosis. To date, antifibrotic treatment of fibrosis represents an unconquered area for drug development, with enormous potential but also high risks. Preclinical research has yielded numerous targets for antifibrotic agents, some of which have entered early-phase clinical studies, but progress has been hampered due to the relative lack of sensitive and specific biomarkers to measure fibrosis progression or reversal. Here we focus on antifibrotic approaches for liver that address specific cell types and functional units that orchestrate fibrotic wound healing responses and have a sound preclinical database or antifibrotic activity in early clinical trials. We also touch upon relevant clinical study endpoints, optimal study design, and developments in fibrosis imaging and biomarkers.**

## The clinical problem

Fibrosis is the excess accumulation of ECM, which results from chronic, nonresolving inflammation. This inflammation triggers a wound-healing process that mitigates inflammatory tissue destruction but also leads to scar tissue formation. In the liver, fibrosis is mainly due to chronic viral hepatitis B or C, autoimmune and biliary diseases, alcoholic steatohepatitis (ASH) and, increasingly, nonalcoholic steatohepatitis (NASH) (1–5). While mild fibrosis remains largely asymptomatic, its progression toward cirrhosis, i.e., replacement of functional parenchyma by scar tissue accompanied by severe architectural and vascular distortion, is the major cause of liver-related morbidity and mortality. Clinical sequelae of cirrhosis are (a) liver synthetic (functional) failure, including failing hemostatic, nitrogen handling, and detoxification systems; (b) portal hypertension with consequent formation of ascites and bleeding esophageal or gastric varices; (c) a high susceptibility to infection; and (d) a high risk to develop hepatocellular carcinoma (HCC) (2). Preventive measures, such as antiviral regimens for hepatitis B or C, are already decreasing the burden of viral cirrhosis and HCC, but other causes, such as NASH (which is linked to obesity and type 2 diabetes) are taking center stage. Moreover, numerous patients present initially in the clinic with advanced fibrosis or cirrhosis, which are largely irreversible. Therefore, antifibrotics that prevent progression toward cirrhosis or induce regression of advanced fibrosis and cirrhosis are urgently needed (6–9).

## Liver fibrosis progression and reversal

Research has delineated key mechanisms and cells that determine fibrosis progression (fibrogenesis) and regression (fibrolysis) (1–19). Notably, liver fibrosis has much in common with fibrosis of other organs, such as lungs and kidneys, leading to a cross-fertilization of research across organ boundaries. The structural components of the fibrotic ECM, the growth factors, cytokines, chemokines, and proteases, as well as central signaling cascades implicated in fibrogenesis and fibrolysis, are nearly identical in these different tissues (18, 20–22). Importantly, fibrosis is no longer considered static, but the result of a continuous remodeling process. Nonetheless, in contrast to kidneys and lungs, the liver has an extraordinary capacity to regenerate, even in advanced fibrosis.

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Fibrosis is intimately linked to wound healing, serving to prevent tissues from disassembly during inflammation, apoptosis, necrosis, and release of lytic enzymes. Fibrosis usually reverses within days to a few weeks following the resolution of tissue damage, as demonstrated in less advanced rodent and human liver fibrosis (2, 8, 9, 23–25). However, the longer the damage persists, often at a low level, the more ECM is deposited. This chronic damage results in increasingly acellular scar tissue and a steep decline of potential reversibility, even after elimination of causative triggers (26, 27). Inefficient fibrolysis is due to several factors: (a) lack of cues for ordered cell repopulation and regeneration due to an atypical ECM and the loss of appropriate cellular context, (b) advanced vascular remodeling with architectural distortion, (c) extensive crosslinking of ECM components such as fibrillar collagen that make proteolytic removal difficult, and (d) the disappearance of cellular elements that digest the scar tissue. Here we discuss the cellular and molecular pathways that promote fibrosis progression and highlight current clinical trials as well as improved methods of monitoring fibrosis.

## Cellular targets and multicellular fibrogenic units

Activated myofibroblasts, representing a spectrum of similar ECM-producing cells that mainly derive from hepatic stellate cells and portal fibroblasts, are the major producers of the fibrotic ECM and the most downstream cellular effectors of liver fibrosis (Figure 1). Very few hepatic myofibroblasts in fibrosis stem from BM-derived fibrocytes (12). Moreover, complete epithelial-mesenchymal transition (EMT) of hepatocytes and bile duct epithelia to myofibroblasts may be a rare event — while an "incomplete" EMT of these cells with acquisition of a fibrogenic phenotype is common (28). Myofibroblasts and their products are primary targets for antifibrotic therapies, which in principle would address all types of fibrosis, including advanced fibrosis.

Importantly, additional cellular elements that are either upstream of the myofibroblasts or tightly linked to fibrogenic activation within cellular units may provide a basis for complementary and more disease-specific antifibrotic approaches. A combination therapy approach may be more effective, given that crosstalk between different cell types generally underlies fibrogenic activation. Conceptually, three major multicellular functional units can be defined according to their constituent cell types: (a) perisinusoidal/vascular — pericytes, i.e., hepatic stellate

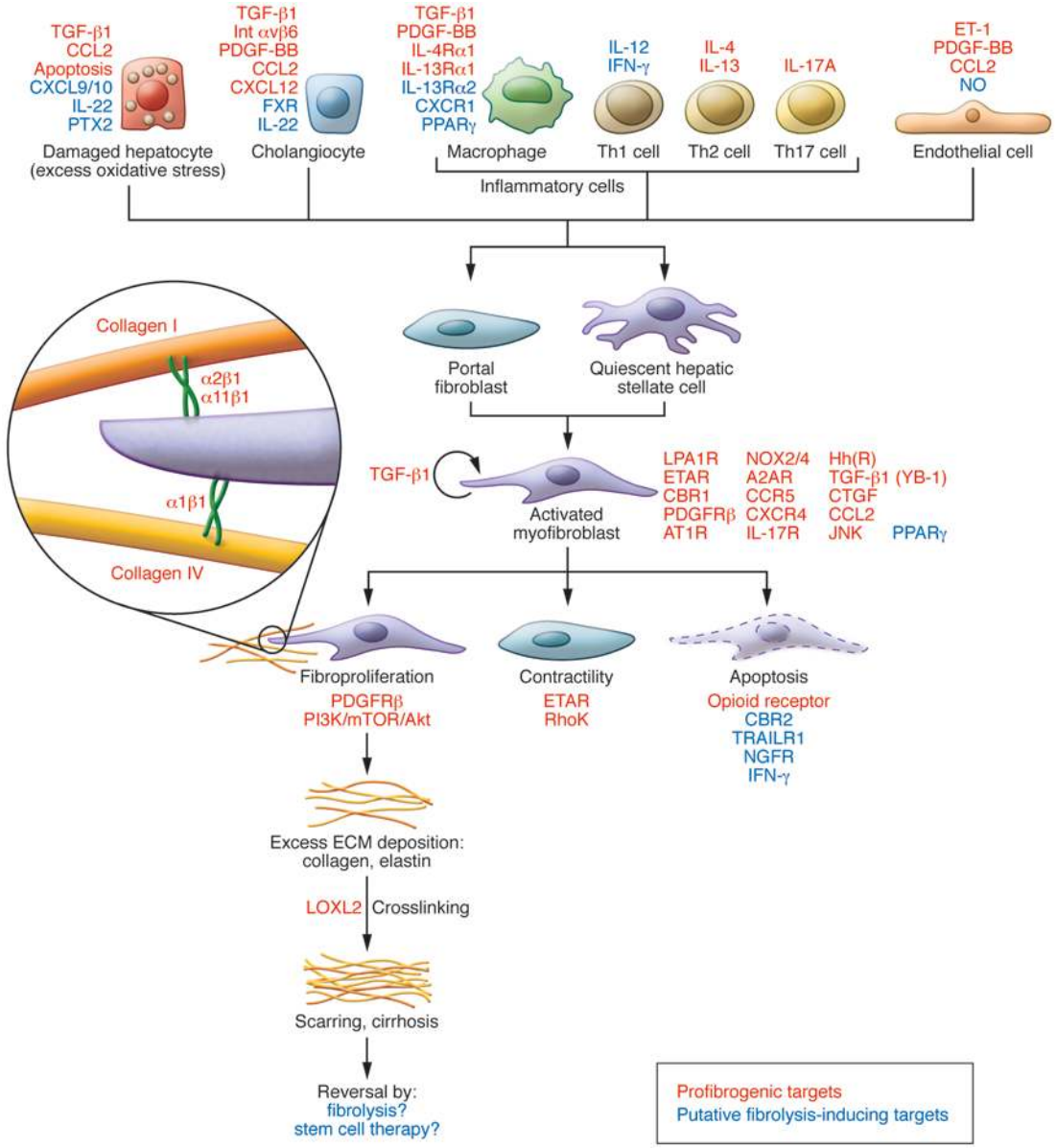


**Figure 1**

Myofibroblasts and their fibrogenic activation. Cells and major factors upstream of quiescent portal fibroblasts and hepatic stellate cells that induce transformation to fibrogenic myofibroblasts. This schematic highlights several major targets to treat liver fibrosis. Notably, the ECM itself can serve as modulator of fibrogenesis and fibrolysis. Thus collagen fibrils become crosslinked by LOXL2, which contributes to the reduced reversibility of advanced fibrosis, and collagen-binding ECM receptors (especially the integrins α1β1, α2β1, and α11β1) confer signals of stress or stress relaxation that either maintain fibrogenic activation or induce fibrolytic activity of the myofibroblasts. Additional minor contributors to fibrogenic activation are not shown here (see text for details). A2AR, adenosine 2A receptor; AT1R, angiotensin 1 receptor; CBR1, cannabinoid receptor 1; ET-1, endothelin-1; ETAR, endothelin A receptor; FXR, farnesoid X receptor; Hh(R), hedgehog (receptor); Int, integrin; LPA1R, lysophosphatidic acid receptor 1; NGFR, nerve growth factor receptor; PTX2, pentraxin 2; TRAILR, TNF-related apoptosis-inducing ligand receptor; YB-1, Y-box binding protein.

cells, liver sinusoidal endothelial cells (LSECs), macrophages/Kupffer cells, and hepatocytes; (b) stromal inflammatory — myofibroblasts, T cells, and macrophages; and (c) portal/periportal — cholangiocytes/ductular cells, portal fibroblasts, and various inflammatory cells (ref. 8 and Figure 2, A–C). Altered interactions within these functional units give rise to the major multicellular fibrogenic pathways.

## Fibrogenic effectors

*Activated myofibroblasts.* Myofibroblasts that derive from both activated hepatic stellate cells and portal fibroblasts are the primary producers of scar tissue (1, 2, 6–22, 29). Notably, myofibroblasts are essential for organ integrity, and their elimination promotes tissue necrosis and inflammation (30). Moreover, myofibroblasts can also contribute to fibrosis regression via release of ECM-

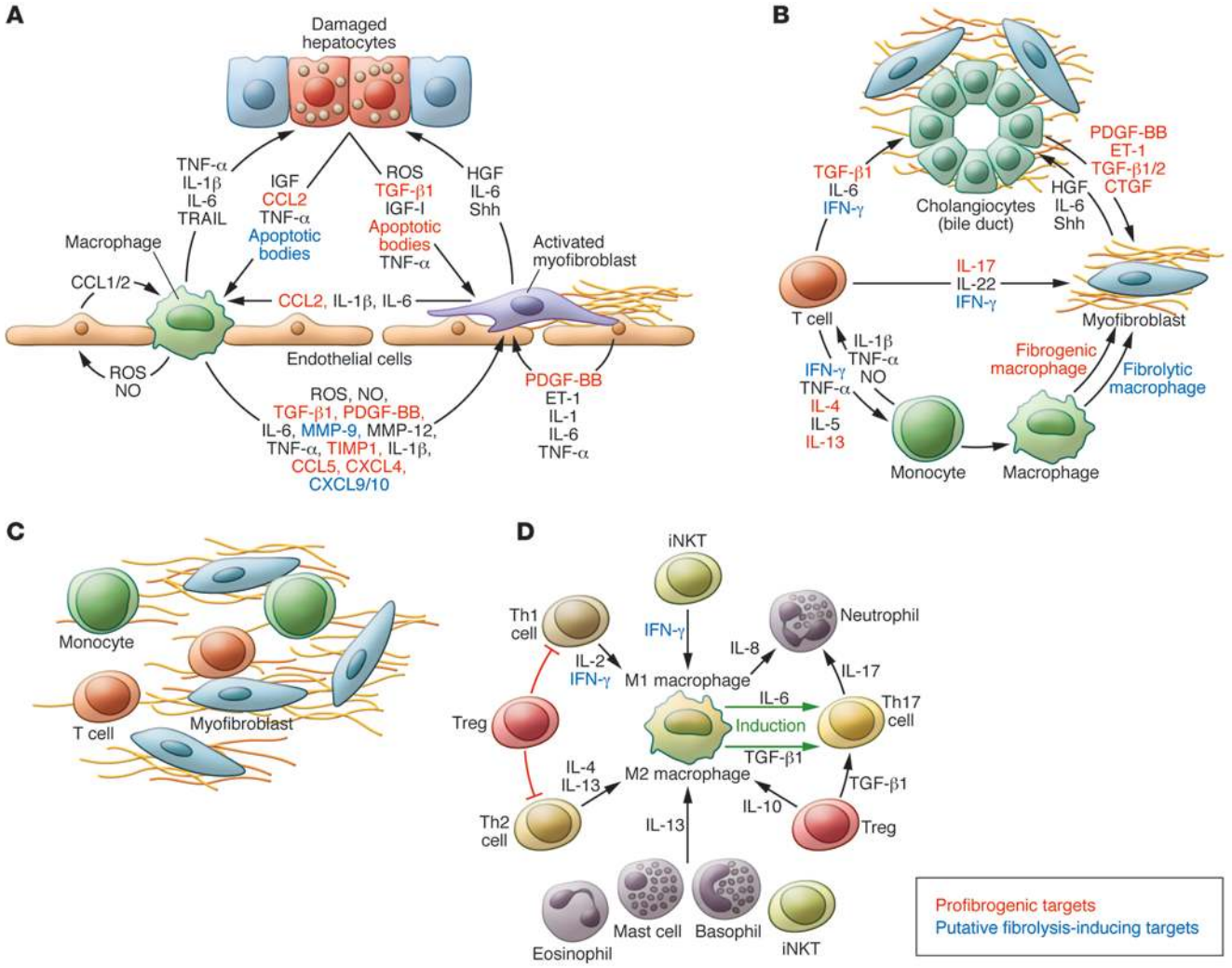


**Figure 2**

Multicellular context of fibrogenesis and fibrolysis. Shown are the postulated major cellular functional units and secreted factors that should be addressed in their complexity when designing effective antifibrotic strategies. (**A**) Vascular unit. (**B**) Biliary unit. (**C**) Inflammatory unit. (**D**) Cells and factors that affect macrophage polarization. Macrophages (and monocytes as macrophage precursors) are major modulators of inflammation and tissue remodeling. Cells and factors that induce either M1 or M2 polarization are also linked to the generation of fibrogenic Th17 cells and neutrophil recruitment. See text for details. **B** and **C** highlight factors not shown in **A** and **B**, respectively. Baso, basophil; EO, eosinophil; Mast, mast cell; PMN, polymorphonuclear neutrophil; TIMP, tissue inhibitor of metalloproteinases.

degrading proteases, when confronted with favorable (e.g., ECM-derived and integrin receptor–mediated) stimuli, in a process called stress relaxation. Stress relaxation is the basis for limiting ECM deposition once the wound is closed: the activated myofibroblasts contract on the accumulated loose collagen matrix, which triggers release of ECM-degrading proteases, mainly MMPs (31, 32). Consequently, treatment strategies should not eliminate myofibroblasts, but rather dampen their fibrogenic activation, confer signals of stress relaxation, and induce fibrolytic enzymes. Accordingly, two rodent studies demonstrated that approximately 50% of activated hepatic stellate cells/myofibroblasts undergo apoptosis during fibrosis reversal, whereas the rest revert to a quiescent phenotype (33, 34). Quiescence can be induced by inhibition of certain fibroblast integrins, cellular receptors that confer mechanical cues in response to ECM attachment (20) with the potential of converting activated to fibrolytic (myo-)fibroblasts (refs. 31, 32, 35, 36 and Figure 1). Specific integrin inhibitors have been developed for cancer therapy, but need better validation for treatment of fibrosis (37, 38). Myofibroblast stress relaxation and resultant amelioration of both fibrogenesis and portal hypertension has been shown in rats by inhibition of Rho kinase, which is downstream of integrin signaling (39).

Several agents that block fibrogenic activation and ECM production by myofibroblasts work well in culture and in some rodent models of liver fibrosis but carry a high risk of unwanted side effects in patients due to a lack of specificity for myofibroblasts. Three major strategies are currently in preclinical development to specifically target the pathogenic function of activated myofibroblasts. First, therapies may address fibrosis-relevant pathways that are upregulated in these myofibroblasts, such as procollagen type I

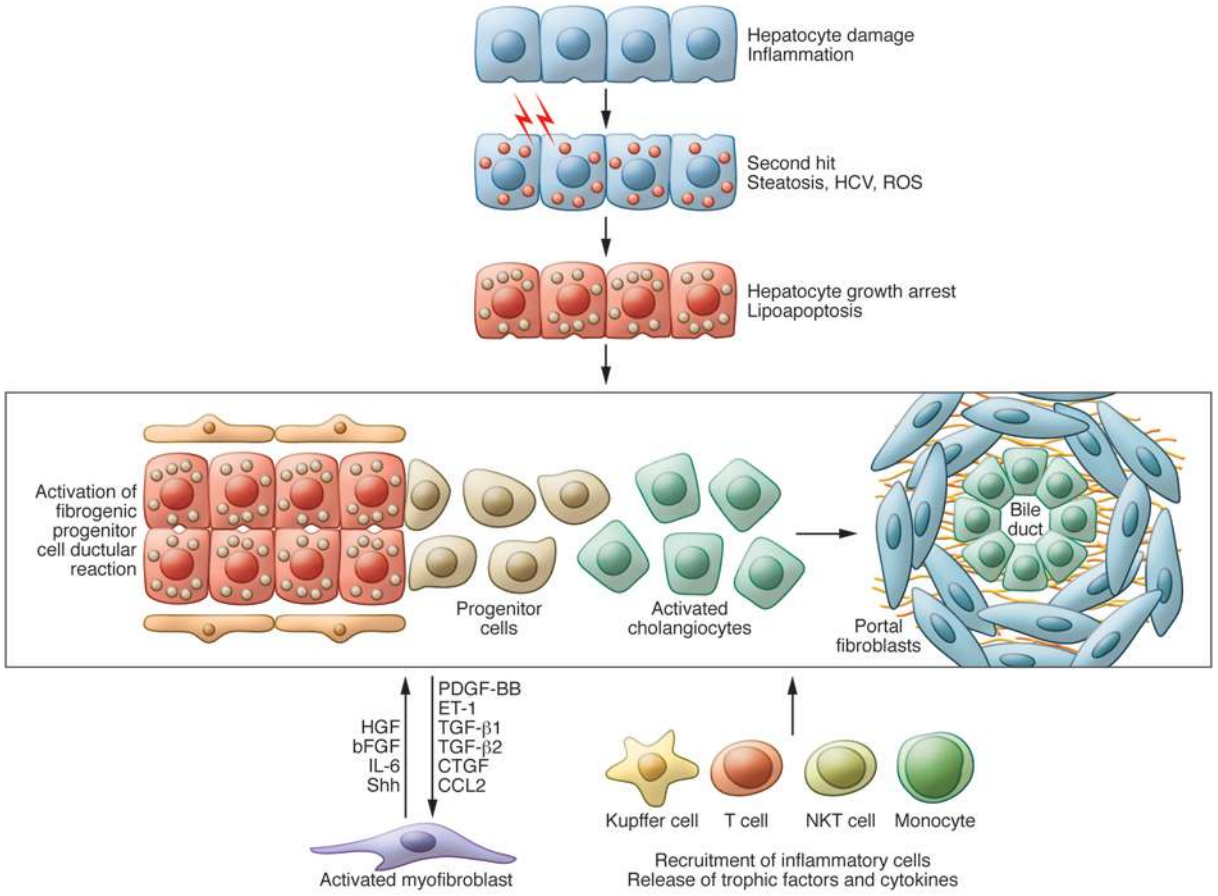


**Figure 3**
Activated cholangiocytes as drivers of fibrosis progression. Activated cholangiocytes are related, if not identical, to biliary progenitor cells. These cells proliferate in active biliary diseases and during massive hepatocyte growth arrest or apoptosis, as in severe NASH, ASH, or viral hepatitis. Biliary progenitor cells are regularly found in more advanced fibrosis (especially Metavir stage F2 or higher). They replicate ductal plate formation by induction of a portal fibrotic matrix via secretion of profibrogenic factors and recruitment and activation of myofibroblasts, and also Kupffer cells and monocytes and other inflammatory cells like T and NKT cells. The recruited myofibroblasts (and the inflammatory cells) secrete factors and ECM components that maintain these fibrogenic units and support their differentiation into more mature biliary structures that are embedded in a collagen-rich ECM.

or other key structural components of the ECM, or block cellular receptors for ECM components and growth factors/chemokines that are upregulated upon fibrogenic activation. Current blockers of collagen synthesis have unwanted off-target effects, but inhibition of upstream fibrogenic signaling, e.g., PDGFRβ, a strong myofibroblast mitogen, with the tyrosine kinase inhibitor imatinib or a more specific PDGFRβ-blocking antibody retarded early but not advanced liver fibrogenesis (40, 41).

A second approach to targeting activated myofibroblasts is to employ refined siRNA delivery techniques, such as liposomal formulations that intrinsically accumulate in liver due to their size, shape, and surface charge, and that deliver cargo to myofibroblasts as well as other liver cell types (42, 43). For example, biliary and parenchymal liver fibrosis was significantly mitigated in mice treated with liposomes loaded with procollagen α1(I) siRNA (44). Finally, the use of ligands specific to receptors on activated myofibroblasts can target drugs or siRNA, thus increasing efficacy and minimizing detrimental off-target effects. Examples supporting this approach in vivo include delivery of IFN via a cyclic PDGFRβ-binding peptide, of a PDGFRβ-specific kinase inhibitor via mannose-6-phosphate (which addresses the IGF-II receptor), and of Hsp47 (which is involved in collagen processing) via vitamin A–coupled liposomes (45–48). Although these therapies would largely need to be given parenterally, such application can be justified in situations in which treatment is likely to be highly effective, e.g., for reversing advanced fibrosis. Moreover, modifications of delivery systems such as pegylation (49) can be used to increase half-lives, permitting once-weekly or once-monthly dosing.

*Damaged hepatocytes*. Ongoing hepatocyte apoptosis or necroptosis, as occurs predominantly in liver diseases characterized by enhanced oxidative and endoplasmic reticulum stress, lysosomal activation, and mitochondrial damage (ASH, NASH), is a strong trigger of fibrogenesis (16, 50). Phagocytosis of apoptotic hepatocytes by myofibroblasts triggers their fibrogenic activation via NADPH oxidase 2 (NOX2) (51) and the JAK/STAT and PI3K/Akt pathways (52). Notably, inhibition of hepatocyte apoptosis by a pan-caspase inhibitor or an antagonist of cathepsin B (a lysosomal trigger of apoptosis) ameliorated (biliary) fibrosis in mice

(53, 54). On the other hand, as mentioned below, engulfment of apoptotic hepatocytes and biliary cells by macrophages can induce their fibrolytic activation.

*Biliary progenitors*. The hallmark of biliary fibrosis is the proliferation of biliary progenitor cells (activated cholangiocytes) that tend to form small clusters or usually nonfunctional bile ductular structures, termed ductular reaction. These cells replicate early developmental programs of ductal plate formation, which includes secretion of several factors that attract and activate hepatic stellate cells/myofibroblasts to proliferate and deposit ECM. This biliary progenitor response is amplified by the surrounding myofibroblasts, but also by inflammatory cells that release molecules that sustain ductular cell viability and proliferation (Figure 3). With the exception of infant fibrosis (biliary atresia, Caroli's disease, congenital hepatic fibrosis) and adult primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), and secondary biliary fibrosis, all liver diseases of other etiologies, once advanced, develop into a portal fibrosis with proliferation of biliary progenitors, especially when excessive hepatocyte apoptosis forces the stem cell niche to produce biliary progenitors. These biliary progenitors are more resistant to enhanced oxidative stress and hepatocyte death, such as induced by ASH, NASH, or severe post-transplant hepatitis C (55–60). Drugs aimed at the biliary fibrogenic progenitors are effective antifibrotic agents in rodent biliary and advanced non-biliary fibrosis. Examples are antagonists to the biliary progenitor-specific integrin αvβ6 (a receptor for fibronectin and tenascin-C, and an activator of latent TGF-β1) (61–63) or inhibition of the hedgehog pathway, which is primarily upregulated in biliary fibrogenesis and in carcinogenesis (56–59, 64, 65). Notably, inhibition of hedgehog signaling suppressed biliary fibrosis and even reversed hepatocellular cancer in phospholipid flippase (*Mdr2*) knockout mice (65).

*LSECs*. Hepatic (neo-)vascularization with LSEC activation and proliferation is tightly associated with perisinusoidal fibrosis (capillarization of the sinusoids) (Figure 1 and Figure 2A). During perisinusoidal fibrosis, activated LSECs contribute to ECM production (including basement membrane components, fibronectin, and interstitial collagen type I), produce cytokines (e.g., TGF-β1 and PDGF-BB) that activate hepatic stellate cells, and secrete factors (e.g., endothelin-1) that contribute to intrahepatic vasoconstriction, which exacerbates portal hypertension in cirrhosis. Conversely, myofibroblasts activate LSEC via secretion of angiogenic factors such as VEGF and angiopoietin-1 (66). Antiangiogenic therapies have mitigated experimental liver fibrosis, mostly in models with a prominent sinusoidal component. However, antifibrotic effects were evident with polykinase inhibitors such as sunitinib and sorafenib that, apart from angiogenic VEGF or FGF receptors on LSECs, also target numerous other cells and kinases involved in proliferation, ECM turnover, and immune regulation (67, 68). This lack of specificity may explain the finding that treatment with anti-VEGF antibody and an antagonist to integrin αvβ3, therapies that inhibit LSEC proliferation (but also affect the proliferation of endothelia of larger vessels) may worsen advanced biliary, perisinusoidal, and interstitial kidney fibrosis (69–71). Moreover, specific inhibition of VEGF mitigates biliary fibrosis progression but retards fibrosis reversal after jejunoileal anastomosis (72). Therefore, as with many other therapies, the antifibrotic efficacy of antiangiogenic therapies is highly context dependent.

*T cells*. $CD4^+$ T cells with a Th2 polarization, which are prevalent in allergies, asthma, or parasite infections, promote fibrogenesis in the liver, lungs, or kidneys (18, 73–75). Th2 cells produce IL-4 and IL-13, which stimulate the differentiation of potentially fibrogenic myeloid cells and (alternatively) activated (M2) macrophages (refs. 73, 76, and Figure 2D). Thus rodents with Th2-dominant T cell infiltration (e.g., in experimental schistosomiasis or in experimental models skewed toward Th2; ref. 77) display rapid fibrosis progression, whereas $CD4^+$ Th1 cells have an antifibrotic effect (78). Accordingly, patients dually infected with HCV and Schistosoma show a 6-fold faster liver fibrosis progression than matched HCV-monoinfected patients (79).

Th17 cells are clear drivers of fibrosis in multiple tissues (80, 81). Th17 cells are induced by a special inflammatory environment, including the cytokines TGF-β1 and IL-6. Th17 cells secrete IL-17A, which drives fibrogenesis directly in myofibroblasts and indirectly via stimulation of TGF-β1 release from inflammatory cells (80, 82).

Regulatory T cells appear to either favor or inhibit fibrogenesis, again in a context-dependent manner. Subsets produce various amounts of the immunosuppressive cytokines IL-10 (potentially antifibrotic) and TGF-β1 (profibrotic). In most settings of chronic inflammation, TGF-β1 prevails.

NK and NKT cells are enriched in the liver and belong to the innate (NK) immune system or the interface between the innate and adaptive (NKT) immune system. In rodent models of liver fibrosis, NK cells repress fibrosis in two ways: (a) by killing early-activated or senescent hepatic stellate cells/myofibroblasts that express NK cell ligands and (b) via production of (antifibrotic) IFN (83). In rodent studies, the effect of invariant NKT (iNKT) cells on liver fibrosis is controversial and modest. At best, iNKTs attenuate early but not late toxin-induced fibrogenesis (84), whereas (variable) NKTs worsened fibrosis in the methionine- and choline-deficient diet NASH model (59). Similar to NK cells, beneficial activity may be explained by killing of hepatic stellate cells/myofibroblasts and IFN secretion, but subsets of iNKT cells can also produce profibrotic IL-13. Notably, iNKT cells protected against diet-induced obesity, insulin resistance, and NASH (85), making them a potential therapeutic target for this common cause of liver fibrosis.

*Monocytes*. Monocytes, which play a key role in inflammation and fibrosis, are also precursors of fibrocytes, macrophages, and dendritic cells and share characteristics with myeloid suppressor cells (86, 87). At the interface of innate and adaptive immunity, monocytes help orchestrate adaptive immune responses, with proinflammatory monocytes ($Ly6C^+Gr1^+$ in mice; $CD14^+CD16^+$ in humans) promoting fibrogenesis (88, 89). Chemokines and their receptors are important in monocyte recruitment and activation, representing attractive targets for fibrosis modulation (16, 90). CCL2 and its receptor CCR2 are central to monocyte recruitment to the inflammatory lesion, and their inhibition ameliorates fibrosis progression in rodent models but retards fibrosis reversal (86). Conversely, the chemokine CXCL9 (and CXCL10) prevents pathological angiogenesis and fibrogenesis via activation of their receptor, CX3CR (91–93). Monocytes are also the precursors of circulating fibrocytes, cells that differentiate into collagen-producing fibroblasts and are related to BM mesenchymal stem cells (12). On the other hand, monocytes are the source of fibrolytic $CD133^+$ cells that home to liver to induce fibrosis reversal after BM transplantation (12, 17). Chemokines and their receptors are important in monocyte recruitment and activation, representing attractive targets for fibrosis modulation.

*Macrophages*. These resident cells derive from circulating monocytes as precursors (partly replenishing the liver specific Kupffer

**Table 1**
Major studies with liver fibrosis as primary endpoint (viral and alcoholic hepatitis and biliary fibrosis)

| Condition | Drug | Study design | Patient population | Results | Year of completion or publication | Phase | No. patients | NCT identifier (reference) |
|---|---|---|---|---|---|---|---|---|
| **HCV (non-antiviral agents)** | | | | | | | | |
| HCV | IL-10 | 1 yr (biopsy) | F, NR | Reduction in fibrosis score; 11/28; 5.0 ± 0.2 to 4.5 ± 0.3 ($P < 0.05$) | 2003 | — | 30 | (132) |
| HCV | Pentoxiphylline (anti–TNF-α) vs. tocopherol | 1 yr; r, db | F | Not reported | 2006 | 3 | 100 | 00119119 |
| HCV | IFN-α2b/R vs. IFN-α2b/R/Viusid | 48 wk; r | F, NR | Improved fibrosis score; 50% (Viusid combination) vs. 37% (non-Viusid), $P = 0.03$ | 2007 | — | 100 | (133) |
| HCV | Losartan | 18 mo; nr, ol | F, NR | No effect (Metavir), significant decrease of profibrogenic gene expression | 2006/2009 | 4 | 20/14 | 00298714 (134) |
| HCV | Farglitazar (PPARγ agonist) | 52 wk; r, db | F, NR | No effect | 2008/2010 | 2 | 225/265 | 00244751 (135) |
| HCV | GS-9450 (pan-caspase inhibitor) vs. plac | 24 wk; nr, db | F, NR | No results reported | 2010 | 2 | 307 | 00874796 |
| HCV | Irbesartan (AT1R antagonist) vs. plac | 2 yr; r, db | F/NR | Pending | 2013 | 3 | 166 | 00265642 |
| HCV | Fuzheng Huayu vs. plac | 48 wk; r, db | F | Pending | 2014 | 2 | 100 | 00854087 |
| HCV/HIV, coinfected | Pio (PPARγ agonist) vs. plac | 48 and 96 wk; r, db | F | Pending | 2013 | 4 | 31 | 00742326 |
| HCV/HIV, coinfected | GS-6624 (anti-LOXL2 mAb) | 24 wk; nr, ol | F | Pending | 2014 | 2 | 30 | 01707472 |
| **HCV (antiviral agents)** | | | | | | | | |
| HCV | Peg–IFN-α2b vs. Peg–IFN-α2b/R vs. IFN-α2b vs. IFN-α2b/R | 24 or 48 wk; r, retro | F, C, NR, SVR | Less worsening of fibrosis (Metavir); 23% (IFN-α2b) vs. 8% (Peg–IFN-α2b/R), $P < 0.001$ | 2002 | 4 | 3,010 | (136) |
| HCV | IFN-γ1b vs. plac | 48 wk; r, db | F, C, NR | No effect | 2003/2007 | 2 | 502 | 00043303 (137) |
| HCV | Peg–IFN-α2a vs. plac | 3.5 yr; r | F, C, NR | No effect | 2008 | — | 1,050 | (138) |
| HCV | Peg–IFN-α2b | 3 yr; r, ol | F, NR | Improved (Metavir); 44/270 (Peg–IFN-α2b) vs. 29/270 (controls) | 2009 | 3 | 540 | 00049842 |
| HCV | Peg–IFN-α2b vs. glycyrrhizic acid | 156 wk; r, ol | F, NR | No effect | 2011 | 3 | 261 | 00686881 |
| HCV/HIV, coinfected | Peg–IFN-α2a/R/HIV antiretroviral therapy vs. HIV antiretroviral therapy alone | 96 wk; r, ol | F, NR | No results reported | 2009 | 3 | 52 | 00122616 |
| HCV/HIV, coinfected | Raltegravir (integrase inhibitor) vs. ritonavir-boosted protease inhibitor | 48 wk; r, ol | F | Pending | 2013 | 2 | 40 | 01231685 |
| **HBV (non-antiviral agents)** | | | | | | | | |
| HBV | SA-B vs. IFN-γ | 6 mo; r, db | F | No effect; fibrosis reduction 36.7% (SA-B) vs 30.0% (IFN-γ) | 2002 | — | 60 | (139) |
| HBV | FG-3019 (anti-CTGF mAb) vs. plac | 45 wk; r, db | F | Pending | 2012 | 2 | 228 | 01217632 |
| HBV/HCV, coinfected | Oltipraz (antiproliferative agent) vs. plac | 24 wk; r, db | F, C | No effect | 2007/2011 | 2 | 83 | 00956098 |
| **HBV (antiviral agents)** | | | | | | | | |
| HBV | Lamivudine vs. plac | 1 yr; r | F | No effect (Knodell); but significant decrease in α-SMA mRNA expression | 2001 | — | 80 | (140) |
| HBV | Adefovir dipivoxil | 3 yr; nr, ol | F, C | Improved in ITT analysis, 52/155; no statistical evaluation | 2009 | 4 | 155 | 00347009 |
| HBV | Tenofovir disoproxil fumate | 5 yr (biopsy); ol | F, C | Improved inflammation (Ishak), 304/348; fibrosis regression, 176/348 ($P < 0.0001$); cirrhosis regression, 71/96 | 2013 | — | 641 | 00117676, 00116805 (112) |
| HBV, reversal | Entecavir | 1 yr; nr, ol | F | Pending | 2017 | 4 | 100 | 01341106 |
| **Other** | | | | | | | | |
| PBC | UDCA vs. plac | 2 yr; db | F, C | No effect (Ludwig) | 1991 | 3 | 146 | (141) |
| PBC | UDCA vs. plac | 4 yr; r, db | F, C | Five-fold lower fibrosis progression rate; 7% (UDCA) vs. 34% (plac) remaining in early stage at 4 yr; 76% (UDCA) vs 29% (plac) | 2000 | 4 | 103 | (142) |
| AH | Candesartan (ACE inhibitor) | 6 mo; r, db | F | Histological improvement; 33.3% vs. 11.6% ($P = 0.020$; Laennec); 26.2% vs. 11.6% ($P = 0.074$; Metavir stage) | 2009–2012 | 1/2–2 | 85 | 00990639 (143) |
| PSC | GS-6624 vs. plac | 96 wk; r, db | F | Pending | 2015 | 2 | 225 | 01672853 |

ACE, angiotensin-converting enzyme; AH, alcoholic hepatitis; AT1, angiotensin II receptor type 1; C, cirrhosis; db, double-blind; F, fibrosis; ITT, intent-to-treat population; NR, non-responders; nr, not randomized; ol, open label; Peg-IFN, pegylated IFN; Pio, Pioglitazone; plac, placebo; r, randomized; retro, retrospective analysis; SVR, sustained viral responders to IFN-based treatment; UDCA, ursodeoxycholic acid. Viusid consists of ascorbic acid, zinc, and glycyrrhizic acid; salvianolic acid B (SA-B) is an ingredient of fuzeng huayu, an herbal antifibrotic.

**Table 2**
Studies with liver fibrosis as primary endpoint (NASH and autoimmune hepatitis)

| Treatment | Study design | Patient population | Results | Year of completion or publication | Phase | No. patients | NCT identifier (reference) |
|---|---|---|---|---|---|---|---|
| **NASH (drug)** | | | | | | | |
| Orlistat (pancreatic lipase inhibitor) vs. 1,400-kcal diet (30% fat) | 36 wk; r, ol | F | No results reported | 2006 | 4 | 50 | 00160407 |
| Pio vs. plac | 6 mo; r, db | | No effect | 2006 | 4 | 55 | 00227110 (144) |
| Pio (PPARγ agonist) vs. plac | 1 yr; r, db | F | Decreased fibrosis progression; 9/31 (29%, Pio.) vs. 6/30 (20%, plac), $P = 0.05$ | 2008 | — | 74 | (145) |
| Metreleptin (leptin analog) | 1 yr; nr, ol | F | No results reported | 2009 | 2 | 10 | 00596934 |
| Pio vs. Vit. E vs. plac | 2 yr; r, db | F | No significant effect; 44% (Pio.) vs. 41% (Vit. E) vs. 31% (plac) | 2009/2010 | 3 | 247 | 00063622 (146) |
| Rosi (PPARγ agonist) vs. plac | 1 and 2 yr; r | F | No effect on fibrosis | 2010 | – | 53 | (147) |
| Pentoxifylline (anti–TNF-α) vs. plac | 1 yr; r, db | F | Improved hepatic steatosis, lobular inflammation and fibrosis | 2010/2011 | 2 | 55 | 00590161 (148) |
| Pentoxifylline vs. plac | 1 yr; r | F | No effect | 2011 | — | 30 | — |
| Rosi vs. Rosi/metformin vs. Rosi/losartan | 48 wk; r, ol | F | No effect on fibrosis | 2011 | — | 137 | (149) |
| High-dose UDCA vs. plac | 1 yr; r, db | F | Significant reduction only of FibroTest | 2011 | 3 | 126 | (150) |
| Metformin | 1 yr; r, db | F | Pending | 2012 | 4 | 80 | 00134303 |
| Liraglutide (GLP-1 agonist) vs. plac | 48 wk; r, db | F | Pending | 2013 | 2 | 50 | 01237119 |
| Pentoxifylline/Vit. E vs. Vit. E | 3 mo (biopsy); r, db | F | Pending | 2013 | 3 | 120 | 01384578 |
| Rosi vs. α-lipoic acid vs. Rosi/α-lipoic acid | 24 wk; r, sb | F | Pending | 2013 | 4 | 26 | 01406704 |
| Losartan (AT1R antagonist) vs. plac | 2 yr; r, db | F | Pending | 2014 | 3 | 214 | 01051219 |
| Obeticholic acid (FXR agonist) vs. plac | 72 wk; r, db | F | Pending | 2014 | 2 | 280 | 01265498 |
| Pio vs. Vit. E vs. plac | 1.5 and 3 yr; r, db | F | Pending | 2014 | 4 | 90 | 00994682 |
| Metreleptin | 1 yr; ol | F | Pending | 2015 | 2 | 20 | 01679197 |
| GS-6624 (anti-LOXL2 mAb; 75 mg and 125 mg) vs. plac | 100 wk; r, db | F | Pending | 2015 | 2 | 225 | 01672866 |
| GS-6624 (anti-LOXL2 mAb; 200 mg and 700 mg) vs. plac | 100 wk; r, db | F,C | Pending | 2015 | 2 | 225 | 01672879 |
| GFT505 (dual PPARα/δ agonist) | 52 wk; r, db | F | Pending | 2015 | 2 | 270 | 01694849 |
| Pio vs. Vit. E vs. Vit. E/Pio vs. plac | 1.5 and 3 yr; r, db | F | Pending | 2015 | 4 | 90 | 01002547 |
| Vit. E/Vit. C vs. plac | 6 mo; r, db | F | No effect | 2003 | — | 49 | (151) |
| Vit. D vs. lifestyle counseling | 2 yr; r, ol | F | Pending | 2014 | 3 | 200 | 01623024 |
| Vit. D3 vs. plac | 48 wk; r, db | F | Pending | 2015 | 2 | 60 | 01571063 |
| Omega-3 (fish) oil vs. plac | 1 yr; r, db | F | No results reported | 2010 | 2/3 | 64 | 00681408 |
| Omega-3 (fish) oil | 18 mo; r, sb | F | Pending | 2013 | 2 | 100 | 00760513 |
| Docosahexaenoic acid | 2 yr; r, db | F | No results reported | 2011 | 1/2 | 60 | 00885313 |
| Eicosapentaenoic acid vs. plac | 48 wk; r, db | F | No results reported | 2011 | 2 | 32 | 00323414 |
| EPA vs. plac | 1 yr; r, db | F | Pending | 2012 | 2 | 243 | 01154985 |
| Diamel vs. plac vs. lifestyle counseling | 52 wk; r, db | F | Pending | 2012 | 3 | 158 | 00820651 |
| Polypill, no biopsy (UE) | 5 yr; r, ol | F | Pending | 2018 | 3 | 1500 | 01245608 |
| **NASH (surgical)** | | | | | | | |
| Bariatric surgery | Meta-analysis of 21 cohort studies | F, C | No clear effect | 2010 | — | 1,643 | (152) |
| **AIH (reversal)** | | | | | | | |
| Corticosteroids/azathioprine | ol, retro | F, C | Decrease of median Knodell score from 14.0 to 1.3, decrease of fibrosis stage from 3.3 to 0.8 | 1997 | — | 8 | (153) |
| **AIH (pediatric)** | | | | | | | |
| Corticosteroids/azathioprine | 4.6 yr | F | Improved fibrosis (Ishak); 14/20 patients (70%, observer 1), 17/20 patients (85%, observer 2) | 2008 | — | 20 | (154) |

AIH, autoimmune hepatitis; AT1R, angiotensin II receptor type 1; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1, Rosi, rosiglitazone; sb, single-blind; UDCA, ursodeoxycholic acid; Vit, vitamin. Diamel is a dietary supplement consisting of lettuce and blueberry extracts, acetylcysteine, arginine, ascorbic acid, cyanocobalamin, zinc sulphate, folic acid, fumaric acid, glycine, calcium pantothenate, L-carnitine, ornithine and pyridoxal; polypill is a combined formulation of aspirin.

cells). M1 macrophages are induced by IFN or IL-12, while IL-4, IL-13, and GM-CSF induce M2 macrophages. Macrophages appear to be fibrogenic during fibrosis progression and fibrolytic during its reversal, but a detailed functional analysis and assignment to M1 or the various M2 subclasses has remained elusive (18, 23, 26). While M1 macrophages are activated in immediate defense against pathogens or detrimental cellular debris, M2 macrophages are generally thought to promote wound healing (i.e., fibrogenesis) and immune suppression (e.g., facilitating cancer growth as tumor-associated macrophages) (18, 94, 95). M2 macrophages respond to IL-4 and IL-13 via IL-4 receptor and IL-13 receptor α1 (with IL-13 receptor α2 serving as negative regulator) and are characterized by unique signal transducers (e.g., Stat6), enzymes (e.g., arginase), or scavenger receptors (e.g., CD206). However, several subtypes of M2 macrophages exist, such as the putatively proinflammatory M2a, and the anti-inflammatory M2b and M2c subtypes, which have ill-defined roles in fibrosis (20, 74). A recent study demonstrated that fibrolytic macrophages in liver fibrosis derive from circulating Ly6C$^{hi}$-expressing monocytes and develop locally into Ly6C$^{lo}$-expressing macrophages with some classical M2 markers and a high expression of fibrolytic MMPs, and this development depends on phagocytic activity (96). Notably, MMP release depends on phagocytosis of apoptotic cells, which is also a driver of biliary fibrosis reversal (23). Given that M1 polarization in liver and adipose tissue enhances insulin resistance and promotes inflammation in NASH, whereas M2 polarization is protective (97), the targeting of macrophage polarization in liver inflammation and fibrosis is an attractive therapeutic option.

### Other relevant molecular targets

Several other molecular targets are of interest, and some have already entered clinical studies. ECM cross-linking, mainly of fibrillar collagen, is largely mediated by lysyl oxidase (LOXL2). LOXL2 likely impedes ECM degradation during fibrosis reversal, and antifibrotic activity has been seen in a small study of CCL4-induced liver fibrosis (98). A humanized antibody that blocks LOXL2 activity is currently assessed in the largest clinical study for liver fibrosis (Tables 1 And 2).

TLRs are sensors of bacteria, viruses, and foreign antigens. TLRs are expressed ubiquitously but are prominent on cells of the innate immune system, creating a proinflammatory environment and activating adaptive immunity to promote pathogen elimination. As the major interface between the gut and systemic circulation, liver cells are equipped with a variety of TLRs that are central to both maintaining immune tolerance and initiating inflammation and repair when confronted with (microbial) danger signals (99). A direct link exists between liver fibrosis and bacterial LPS, and activation of its receptor TLR4. LPS enters the portal hepatic circulation in conditions of enhanced intestinal permeability, such as in ASH, NASH, and other intestinal and liver diseases. LPS upregulates chemokine secretion of monocytes and macrophages/Kupffer cells and downregulates the inhibitory TGF-β1 pseudoreceptor Bambi, which cumulatively sensitizes hepatic stellate cells/myofibroblasts to fibrogenic activation (99, 100). Prevention of excessive TLR4 activation or inhibition of TLR4 are therefore attractive strategies to inhibit fibrogenesis. Currently only the parenteral TLR4 antagonist, eritoran tetrasodium, is being studied for the treatment of sepsis (101). Other interesting but little explored targets include TLR3, a double-stranded RNA sensor whose activation by polyI:C attenuates liver fibrosis via activation of NK cells (102), and TLR9, a receptor for double-stranded bacterial DNA that enhances fibrogenic immune activation via release of CCL2 (103). In addition, inhibitors of broadly expressed chemokine systems other than CCR2/CCL2, mainly CXCL4 and CCL5 (and their receptors CXCR4 and CCR5, respectively) on myofibroblasts, T cells, and macrophages, have been shown to attenuate liver fibrosis (104–106). Furthermore, the recent explosion of data related to microRNAs (miRs) has uncovered miRs that inhibit (miR-29b) or promote fibrogenesis (miR-199, miR-200, and others) (107–109). While these miRs appear to have some specificity for myofibroblasts, their efficient in vivo delivery poses a problem.

TGF-β and, to a lesser degree, its downstream mediator, connective tissue growth factor (CTGF), are potent profibrogenic cytokines for hepatic stellate cells/myofibroblasts (1, 2, 6, 9, 10, 18, 19). However, their general and untargeted inhibition poses risks, especially for TGF-β–neutralizing agents, given that this cytokine is central to cellular differentiation, immune regulation (dampening excessive T cell activation), and regulated wound healing, such as in vascular plaque stabilization in atherosclerosis (110).

### Preclinical proof of concept

Before entering clinical studies, best preclinical proof of antifibrotic activity needs to be obtained in complementary rodent models that reflect different aspects of human liver fibrosis (6). Moreover, drug testing in cultures of precision-cut human liver slices obtained from operations permit a first translation toward the human in vivo system (111).

### Combination therapies

Combination therapies that address liver fibrosis in a multipronged approach hold much promise for future treatment, ideally targeting interactions between cells, soluble mediators, the ECM and its receptors, and/or relevant intracellular signaling. Combinations of targeted antifibrotic agents have yet to be thoroughly tested in preclinical studies. Significant expense and effort will be required to rigorously validate combinations at different doses and in several rodent fibrosis models. However, combinations of specific drugs can be anticipated that interfere with fibrogenesis, induce fibrolysis, or address different cell types.

Clinical development of combination therapies that could guarantee thorough efficacy and low toxicity is only feasible with the advent of improved noninvasive biomarkers and technologies to measure fibrosis, and especially fibrogenesis. Moreover, the necessary personalized approach to the patient with liver fibrosis or cirrhosis will only be possible with such biomarkers, permitting the adjustment of different medications and their dose according to a readily measurable treatment effect.

### Testing antifibrotics in clinical trials

Recent clinical trials with efficient causal therapy have demonstrated reversibility of advanced liver fibrosis. Perhaps the best example is a study of 348 patients with chronic hepatitis B who were treated with the potent antiviral tenofovir (112). After five years, regression of fibrosis was observed in 91% of patients with significant fibrosis at study entry. Only 12 of 252 patients (5%) showed fibrosis progression, while 71 of the 96 patients (74%) with cirrhosis at baseline were no longer cirrhotic at year five. Moreover, all but one of these individuals had at least a two-unit reduction (out of a possible total of six units) in Ishak fibrosis score at year five, a difference that strongly rules out biopsy sampling variability.

**Table 3**
Studies in pulmonary, renal, and other fibrosis with fibrosis as primary endpoint

| Drug | Study design | Results | Year of completion or publication | Phase | No. patients | NCT identifier (reference) |
|---|---|---|---|---|---|---|
| **Pulmonary fibrosis** | | | | | | |
| Etanercept (anti–TNF-α) vs. plac | 48 wk; r, db | No effect | 2005/2008 | 2 | 88 | 00063869 (155) |
| NAC (antioxidant) vs. plac | 1 yr; r, db | Reduction in FVC and $DL_{CO}$ in NAC group, no change in mortality | 2005 | 1/2 | 182 | (156) |
| Tetrathiomolybdate (antiproliferative) | nr, ol | No results reported | 2005 | 1/2 | 20 | 00189176 |
| IFN-α oral lozenge | 1 yr; nr, ol | Minimal/no progression (HRCT), 12/18 patients | 2007 | 2 | 18 | 01442779 |
| Thalidomide (anti-TNF) | 1 yr; nr, ol | No results reported | 2007 | 2 | 19 | 00162760 |
| Bosentan (dual $ET1_AR$ and $ET1_BR$ antagonist) vs. plac | 1 yr; r, db | Worsening PFT; two-thirds exhibited reduction in FVC (>10%), $DL_{CO}$ (>15%), $O_2$ saturation (>4%) | 2005/2008 | 2/3 | 158 | 00071461 |
| Bosentan vs. plac | 12 mo, 21 mo, and 3 yr (biopsy); r, db | No significant effect | 2010/2011 | 3 | 616 | 00391443 |
| Losartan (AT1R) | 1 yr; ol | Stable or improved FVC in 12/17 patients | 2012 | — | 20 | (157) |
| Imatinib (kinase inhibitor) vs. plac | 92 wk; r, db | No effect | 2010 | 2/3 | 120 | 00131274 (158) |
| Ambrisentan ($ET1_AR$ antagonist) vs. plac | 92 wk; r, db | Terminated due to lack of efficacy | 2012 | 3 | 600 | 00768300 (159) |
| Pirfenidone (anti–TGF-β, anti–TNF-α, anti–IL-1β) vs. plac | 72 wk; r, db | Study 004: reduced decline in FVC ($P = 0.001$); –8.0% vs. –12.4% in high-dose pirfenidone group at week 72 | 2008 | 3 | 435 | 00287716 (160) |
| Pirfenidone (anti–TGF-β, anti–TNF-α, anti–IL-1β) vs. plac | 72 wk; r, db | Study 006: difference in FVC change at week 72 not significant ($P = 0.501$) | 2008 | 3 | 344 | 00287729 (160) |
| Pirfenidone vs. plac | 52 wk; r, db | Significant worsening of FVC; +0.009 L (Pirfenidone) vs. –0.16 (plac), $P = 0.0416$ | 2010 | 3 | 275 | (161) |
| BIBF1120 (nintendanib, multi-RTK inhibitor and angiokinase inhibitor; low dose) vs. plac | 1 yr; r, db | FVC decline 68.4% reduction with BIBF1120 vs. plac ($P = 0.01$); lower incidence of acute exacerbations vs. plac per 100 patient-years ($P = 0.02$) | 2010/2011 | 2 | 432 | 00514683 (162) |
| BIBF1120 vs. plac | 52 wk; r, db | Pending | 2013 | 3 | 551 | 01335477 |
| BIBF1120 vs. plac | 52 wk; r, db | Pending | 2013 | 3 | 515 | 01335464 |
| BIBF1120 | 5 yr; ol | Pending | 2015 | 2 | 20 | 01417156 |
| BIBF1120 | 3 yr; nr, ol | Pending | 2015 | 2 | 198 | 01170065 |
| CNTO888 (anti-MCP1/CCL2 mAb) vs. plac | 74 wk; r, db | No results reported | 2012 | 2 | 126 | 00786201 |
| QAX576 (anti–IL-13 mAb) | 4 wk; nr, ol | No results reported | 2009 | 2 | 52 | 00532233 |
| QAX576 vs. plac | 1 yr; r, db | Pending | 2013 | 2 | 40 | 01266135 |
| SAR156597 (anti–IL-4/13 mAb) vs. plac | 6 mo; r, db | Pending | 2014 | 1/2 | 24 | 01529853 |
| Octreotide (somatostatin mimetic) | 1 yr; nr, ol | Pending | 2012 | 1/2 | 25 | 00463983 |
| CC-930 (JNK inhibitor) vs. plac | 4 wk; r, db | Pending | 2013 | 2 | 28 | 01203943 |
| STX-100 (anti-αVβ6 mAb) vs. plac | 24 wk; r, db | Pending | 2013 | 2 | 32 | 01371305 |
| FG-3019 (anti-CTGF mAb) | 109 wk; ol | Pending | 2014 | 2 | 84 | 01262001 |
| **Cystic fibrosis (lung)** | | | | | | |
| SB656933 (CXCR2 antagonist) vs. plac | 28 d; r, db | No results reported | 2010 | 2 | 100 | 00903201 |
| Simvastatin (HMGR inhibitor) vs. plac | 12 wk; r, db | Pending | 2012 | 1/2 | 120 | 01092572 |
| **Renal fibrosis** | | | | | | |
| Pirfenidone | 1 yr; ol | Decreased GFR in focal glomerulosclerosis and nephrotic syndrome; not significant | 2008 | 2 | 21 | 00001959 |
| Everolimus (mTOR inhibitor)/EC-MPA/corticosteroids vs. cyclosporine A/EC-MPA/corticosteroids | 12 mo; r, ol | Pending | 2012 | 3 | 235 | 01079143 |
| Pioglitazone vs. plac | 48 wk; r, db | Pending | 2012 | 4 | 160 | 00745225 |
| **Skin fibrosis** | | | | | | |
| Pravastatin (HMGCoAR inhibitor) | 1 yr; nr, ol | Pending | 2013 | 2 | 40 | 01268202 |
| **Myelofibrosis** | | | | | | |
| GS-6624 (anti-LOXL2 mAb) | 24 wk; r, ol | Pending | 2013 | 2 | 54 | 01369498 |
| **EE** | | | | | | |
| QAX576 (anti–IL-13 mAb) vs. plac | 13 wk; r, db | Pending | 2012 | 2 | 25 | 01022970 |

AT1R, angiotensin II receptor type 1; $DL_{CO}$, diffusing capacity of the lungs for carbon monoxide; EC-MPA, enteric-coated mycophenolate sodium; EE, eosinophilic esophagitis; $ET1_AR$, ET-1 receptor type A; FVC, forced vital capacity; GFR, glomerular filtration rate; HMGCoAR, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; HRCT, high-resolution computed tomography; NAC, N-acetylcysteine; PFT, pulmonary function test; RTK, receptor tyrosine kinase. "Skin" indicates radiation-induced cutaneous and subcutaneous fibrosis.

**Table 4**
Drugs in clinical trials for other indications that have antifibrotic potential for liver fibrosis

| Type of agent | Drug | Manufacturer | Disease target or clinical endpoint | Year of completion | Phase | NCT identifier [no. studies] (reference) |
|---|---|---|---|---|---|---|
| Anti–TGF-β mAb | Fresolimumab (GC1008) | Genzyme | IPF | 2008 | 1 | 00125385 |
| Anti–TGF-β mAb | Fresolimumab (GC1008) | Genzyme | FSGS[A] | 2014 | 2 | 01665391 |
| Anti-αVβ6 mAb | STX-100 | Biogen-Stromedix | IPF[A] | 2013 | 2 | 01371305 |
| Anti-CTGF mAb, anti-CTGF antisense DNA | FG-3019 | FibroGen | T2DM (DN[A]) | 2010 | 2 | 00913393 |
| Anti-CTGF mAb, anti-CTGF antisense DNA | FG-3019 | FibroGen | IPF[A] | 2014 | 2 | 01262001 |
| Anti-CTGF mAb, anti-CTGF antisense DNA | EXC001 (anti-CTGF antisense DNA) | Pfizer-Excaliard | Scar prevention | 2010–2012 | 2 | [4] |
| PRM | PRM-151 (recombinant pentraxin-2) | Promedior | IPF | 2012 | 1 | 01254409 (163) |
| PRM | PRM-151 (recombinant pentraxin-2) | Promedior | Glaucoma | 2012 | 2 | 01064817 |
| Anti–IL-4/13 mAb | SAR156597 | Sanofi-Aventis | IPF[A] | 2014 | 2 | 01529853 |
| Anti–IL-13 mAb | QAX576 | Novartis | IPF, EE, CD, AR | 2009–2013 | 2 | [4] |
| Anti–IL-13 mAb | QAX576 | Novartis | Asthma | 2012–2013 | 1/2 | [4] |
| Neurochemical receptor | S-777469 | Shionogi Pharmaceuticals | AD | 2008–2009 | 1/2–2 | — |
| Neurochemical receptor | LH-21 (peripherally active CB1R antagonist) | — | Obesity | — | Preclinical | — |
| Neurochemical receptor | CB2R agonist (HU-308, JWH 133, A-836339, BML-190, AM1241) | — | — | — | Preclinical | — |
| Neurochemical receptor | Naltrexone (opioid receptor antagonist) | Dupont | Liver fibrosis | — | Preclinical | — |
| Anti–IL-17A, anti–IL-17R | Secukinumab (AIN 457, anti–IL-17A mAb) | Novartis | PP, AS, PA, RA, asthma, uveitis, CD | — | 2–3 | [45] (164) |
| Anti–IL-17A, anti–IL-17R | Ixekizumab (LY2439821, anti–IL-17A mAb) | Eli Lilly | PP, PA, RA | — | 2–3 | [9] (164) |
| Anti–IL-17A, anti–IL-17R | Brodalumab (AMG827, anti–IL-17RA) | Amgen-AstraZeneca | PA, PP, CD, RA, asthma | — | 2–3 | [13] (164) |
| Anti–IL-17A, anti–IL-17R | RG4934 (humanized anti–IL-17A mAb) | Roche | PA | No information available | 1 | No information available |
| Anti–IL-17A, anti–IL-17R | NI-1401 (RG7624, anti–IL-17A/F mAb) | Roche | Autoimmune disease | No information available | 1 | No information available |
| Anti–IL-17A, anti–IL-17R | SCH 900117(anti–IL-17A mAb) | Schering-Plough | RA | | | |
| Anti–IL-17A, anti–IL-17R | ABT-122 (anti–IL-17A and TNF mAb) | Abbott | RA | | | |
| Hh or Hh(R) (SMO) antagonist | Vismodegib (GDC-0449, Hh(R) blocker) | Genentech | Various cancers; MM | — | 1–2 | [38] |
| Hh or Hh(R) (SMO) antagonist | LY2940680 (SMO antagonist) | Eli Lilly and Company | NSCLC, MB, other cancers | — | 1–1/2 | [5] |
| CCR5 antagonist | TBR 652 (small-molecule inhibitor) | Tobira Therapeutics | HIV | Completed | 1/2–2 | [3] (165) |
| CCR5 antagonist | Vicriviroc (SCH-D, SCH 417690, small-molecule inhibitor) | Schering-Plough | HIV | Completed | 1–3 | [11] (165) |
| CCR5 antagonist | Aplaviroc (GW873140, small-molecule inhibitor) | GlaxoSmithKline | HIV | — | 2–3 | [8] (165) |
| CCR5 antagonist | Maraviroc (small-molecule inhibitor) | Pfizer | HIV, ED, cancer | Approved | 1–4 | [104] (165) |
| CCR5 antagonist | INCB009471 (small-molecule inhibitor) | Incyte | HIV | 2007 | 1/2 | 00393120 (165) |
| CXCR4 antagonist, anti-CXCR4 mAb | BKT140 (small-molecule inhibitor) | Biokine Therapeutics | MM, leukemia | 2010 | 1/2 | 01010880 (166) |
| CXCR4 antagonist, anti-CXCR4 mAb | TG-0054 (small-molecule inhibitor) | TaiGen Biotechnology | MM, leukemia | Completed | 1–2 | [3] (166) |
| CXCR4 antagonist, anti-CXCR4 mAb | Plerixafor (AMD3100, small-molecule inhibitor) | Genzyme | MM, NHL, ML | — | 1–4 | [94] (166) |
| CXCR4 antagonist, anti-CXCR4 mAb | POL6326 (small-molecule inhibitor) | Polyphor Ag | MM, leukemia | 2015 | 1/2 | 01413568 (166) |
| CXCR4 antagonist, anti-CXCR4 mAb | MDX-1338 (BMS-936564, anti-CXCR4 mAb) | Bristol-Myers Squibb | MM, ML | 2014 | 1 | [2] (166) |
| CXCR3 antagonist | SCH 546738 (small-molecule antagonist) | Merck | RA | — | 1 | — |
| CXCR3 antagonist | AMG487 (T487, small-molecule antagonist) | Amgen | RA | — | 1–2 | — |
| NOX inhibitor | NOX inhibitor (GKT137831, dual NOX1/4 inhibitor) | Genkyotex | Healthy individuals | Completed | 1 | — |
| Copaxone | Copaxone | Teva Pharmaceuticals | MS | — | 2–4 | [44] |
| Adiponectin, AMPK | Recombinant adiponectin/AdipoR1/2 and AMPK agonist | — | Liver fibrosis | — | Preclinical | (167) |
| YB-1 | TGF-β pathway inhibitor | — | Liver fibrosis | — | Preclinical | — |
| LPA1R | AM152 (myofibroblast recruitment inhibitor) | Amira Pharmaceuticals | IPF, liver fibrosis | — | 2, — | — |
| Anti-Th17 MMP inducer | Halofuginone | Collgard | Kaposi sarcoma | Completed | 1, 2 | [2] (168) |
| Adenosine receptors | ZM241385 (adenosine receptor A2A antagonist) | — | Liver fibrosis | — | Preclinical | (169) |
| miR | miR-29 | Mirage | Cardiac fibrosis | 2008 | Preclinical | — |
| miR | Anti–miR-21 | Regulus | Cardiac and renal fibrosis | — | Preclinical | — |
| miR | Anti–miR-122 (SPC3649, Miravirsen) | Santaris Pharm A/S | Hepatitis C | — | 1–2 | [5] |

[A]Fibrosis as secondary endpoint. AD, atopic dermatitis; AdipoR, adiponectin receptor; AR, allergic rhinitis; AS, ankylosing spondylitis; CB1(2)R, cannabinoid receptor type 1(2); CD, Crohn's disease; DN, diabetic nephropathy; ED, endothelial dysfunction; EE, eosinophilic esophagitis; FSGS, focal segmental glomerulosclerosis; IPF, idiopathic pulmonary fibrosis; LPA1R, lysoposphatidic acid receptor 1; MB, medulloblastoma; ML, myeloid leukemia; MM, multiple myeloma; NHL, non-Hodgkin's lymphoma; NSCLC, non-small cell lung cancer; PA, psoriatic arthritis; PP, plaque psoriasis; PRM, recombinant human serum amyloid P; RA, rheumatoid arthritis; SMO, smoothened; T2DM, type 2 diabetes mellitus; YB-1, Y-box binding protein-1. Copaxone is a T cell differentiation–inhibiting glatiramer acetate mixture of oligopeptides, and a TLR9 agonist.

**Table 5**
Stem cell therapies for liver fibrosis and cirrhosis

| Cell type | Intervention | Patient population | Evidence of efficacy | Phase | No. patients | NCT identifier (reference) |
|---|---|---|---|---|---|---|
| UC-MSC | ol | C | Improved liver function, MELD, and reduced ascites | — | 45 | (Summarized in ref. 170) |
| UC-MSC | ol | C | Improved liver function, MELD, and increased survival | — | 43 | (Summarized in ref. 170) |
| BM-MSC | ol | C | Improved liver function and MELD | — | 158 | |
| BMNC | ol | C | Improved ascites and MELD | — | 40 | |
| $CD34^+$ | ol | C | Improved MELD | — | 4 | |
| PBMC from G-CSF | ol | C | Improved liver function, reduced Child-Pugh score | — | 40 | |
| $CD133^+$ BMSCs | ol | C | Increased liver volume after liver resection | — | 6 | |
| BM-MSC | r, ol | C, alcohol | Pending | 2 | 12 | 01741090 |
| BM-MSC | r, ol | C, HBV | Pending | 2 | 240 | 01728727 |
| UC-MSC | r, ol | C, PBC | Pending | 1/2 | 100 | 01662973 |
| UC-MSC | r, sb | C, reversal | Pending | 1/2 | 200 | 01233102 |
| UC-MSC | r, ol | C, reversal | Pending | 1/2 | 45 | 01220492 |
| HMB-MSC | r, ol | F/C, reversal | Pending | 1/2 | 50 | 01483248 |
| BM-MNC plus $CD133^+$ | r, db | C, reversal | Pending | 1/2 | 30 | 01120925 |
| PBSC | r, ol | C, HBV | Pending | 1/2 | 20 | 01728688 |
| ABMSC plus portal hypertension surgery | nr, ol | C | Pending | 2/3 | 50 | 01560845 |

ABMSC, autologous BM stem cell; BM-MNC, BM mononuclear cell; BM-MSC, BM mesenchymal stem cell; BMNC, blood mononuclear cell; HMB-MSC, human menstrual blood–derived mesenchymal stem cell; PBSC, autologous peripheral blood stem cell; UC-MSC, umbilical cord–derived mesenchymal stem cell.

While some additional human studies also suggested antifibrotic activities of tenofovir, others failed to show an effect. Tables 1 and 2 list past and current clinical studies with liver fibrosis as primary endpoint, and Supplemental Table 1 (supplemental material available online with this article; doi:10.1172/JCI66028DS1) shows studies with liver fibrosis as a secondary endpoint. Table 3 highlights clinical studies for other fibrotic diseases that are relevant for liver. Clinical studies targeting major multicellular fibrogenic pathways are shown in Table 4. We also summarize trials employing stem cells, an approach that is attractive in combination with pharmacological therapies (Table 5). Notably, development efforts have largely focused on extracellular targets because intracellular targets are less accessible and tend to lack specificity for the fibrogenic cells.

*Optimal selection and stratification of patients*. Subjects should be matched according to etiology, age, gender, signs of the metabolic syndrome, medications, and risk factors such as alcohol or tobacco consumption (7–9). Preferably, patients should be in an intermediate fibrosis stage, where dynamic changes of fibrosis are best detectable. Patients with chronic HCV infection following liver transplant are considered a preferred study population because up to 30% experience an accelerated fibrosis progression to cirrhosis within three to five years (60). Patients should be further stratified according to their genetic risk to progress to cirrhosis (7, 8, 60, 113).

## Assessing fibrosis

Assessment of fibrosis progression is far more difficult for liver than for lungs or kidneys because transaminases do not correlate with fibrosis or fibrogenesis and liver function parameters such as albumin or prothrombin time (protein synthesis) are usually only altered in cirrhosis. Liver biopsy remains the standard for studies with antifibrotics. However, liver biopsy is invasive and risky (2, 6, 8, 60, 114) and prone to considerable sampling error, and its interpretation is subject to interobserver variability. Even in well-stratified cohorts, given the usually slow fibrosis progression, conventional fibrosis staging (Metavir, Ishak) may require approximately 200 patients studied over a period of two to three years to detect a 20%–30% difference in fibrosis between treatment groups. However, by including current surrogates of fibrosis progression, it appears feasible to conduct proof-of-concept trials in approximately 100 patients within 12 months or less.

*Refined liver biopsy readouts*. Predictive value may be improved using dynamic biopsy-derived parameters, such as semiquantification of activated α-SMA–positive myofibroblasts and the fibrogenic cytokine TGF-β after immunostaining (115), or quantitative PCR quantification of transcripts that are related to fibrogenesis or fibrolysis (116).

*Radiological imaging*. Conventional and contrast ultrasonography, computerized tomography, and MRI, PET, single-photon emission computerized tomography, and diffusion-weighted MRI cannot differentiate fibrosis stages. However, magnetic resonance texture analysis, which requires sophisticated instrumentation and software, may permit semiquantitative fibrosis assessment (4, 8).

*Elastography*. Ultrasound elastography (UE) and axial radiation force imaging (ARFI) measure hepatic stiffness and elasticity. These techniques sample a 100-fold-larger volume than biopsy and can differentiate mild (Metavir F0–F1) from significant fibrosis (F2–F4) and cirrhosis (F4), with diagnostic accuracies (area under receiver operating characteristics [AUROC] curves) around and above 0.90, which is considered good (8, 114, 116). Magnetic resonance elastography assesses the whole liver and may be superior to UE/ARFI, but is not generally available, and studies are

small (8, 117). UE is useful for initial stratification of patients according to fibrosis stage.

*Serum fibrosis markers*. More than 2,000 studies in the last five years have employed serological markers to assess liver fibrosis, mostly in patients with HCV (2, 6, 8, 60, 118–121). These markers reflect liver function (indirect markers), are related to matrix metabolism (direct markers), or both. The best marker panels show AUROCs around 0.8–0.85 to differentiate between no/mild fibrosis (Metavir F0–F1) and moderate/severe fibrosis (F2–F4). Fibrosis markers have almost exclusively been validated as predictors of fibrosis stage, while especially the direct parameters may rather reflect the dynamics of fibrogenesis and/or fibrolysis (2, 8). Recent studies suggest that certain marker combinations such as the (indirect) Fibrotest (122) and the (direct) enhanced liver fibrosis (ELF) test can predict hard endpoints. Thus ELF was superior to fibrosis stage, the Child-Pugh or the Model for End-Stage Liver Disease (MELD) score, to predict hepatic decompensation or death in long-term, retrospective follow-up studies of patients with advanced chronic HCV or PBC (123–125).

*Combination of methods*. The combination of serum fibrosis markers with elastography increases diagnostic accuracy, permitting a clear allocation to either no/mild (F0–F1) or significant (F2–F4) fibrosis in 70% of patients (8, 114).

*Measurement of portal hypertension and quantitative liver function*. The hepatic vein pressure gradient (HVPG) is an excellent predictor of decompensation or death in patients with cirrhosis (126). A non-invasive alternative, the hepatic vein arrival time of an injected ultrasound contrast agent, needs further validation (127).

Tests that measure the metabolic capacity of the liver, such as demethylation of ingested methacetin and quantification of the exhaled metabolite $^{13}CO_2$, correlate inversely with the severity of liver inflammation and fibrosis, and the results of such tests can complement antifibrotic drug trials (128).

*Quantitative imaging of liver fibrosis and fibrogenesis*. Methods that employ a small molecular ligand for fibrillar collagen, elastin, or a cell-associated molecule coupled to a radio-imaging or MRI agent are in development (4, 8). Examples include an elastin-specific MRI probe for imaging of fibrosis (129) and probes for quantifying fibrogenic cells via the cholangiocyte integrin αvβ6 or the myofibroblast-specific PDGFRβ (4, 8). When improved, such methodology could serve as a novel gold standard for the assessment of fibrosis/fibrogenesis and permit short-term testing of potential antifibrotics before and after a single dose of the drug.

*Novel biomarkers*. Apart from ongoing efforts to find and validate better serum markers of fibrosis, fibrogenesis, and fibrolysis (4, 8), three methodologies will likely become relevant for antifibrotic drug trials: (a) urinary assessment of proteolytic activities in the fibrotic liver could be monitored after injection of mass-encoded protease-sensitive peptides conjugated to nanoparticles and multiplexed detection of cleavage products by mass spectrometry (2, 130); (b) membrane microparticles, which are shed from activated or apoptotic cells, can be quantified in the bloodstream via their cell-specific surface molecules; microparticles represent a novel set of quantitative diagnostic markers to monitor cell-specific activation in liver inflammation and fibrosis (3, 131); and (c) circulating miRs that can reflect liver-specific pathology, including hepatocyte differentiation and activation, cancer growth, and liver fibrosis (109).

## Conclusions

We have gained remarkable insight into the cellular and molecular mechanisms of liver fibrosis and reversal, and even reversal of cirrhosis appears feasible in preclinical models. Currently, the field has progressed toward clinical translation. As antifibrotics address mechanisms that are embedded in a complex multicellular network, their efficacy is predicted to be context dependent. Combination therapies hold most promise, but their development and use require a personalized medicine approach that depends on the development and validation of novel noninvasive markers and techniques to quantify liver fibrosis and especially fibrogenesis.

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Address correspondence to: Detlef Schuppan, Institute of Molecular and Translational Medicine and Department of Medicine I, Johannes Gutenberg University, Langenbeckstr.1, 55131 Mainz, Germany. Phone: 49.6131.177356; Fax: 49.6131.177357; E-mail: detlef.schuppan@unimedizin-mainz.de.

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