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Review Series

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Evolving therapies for liver fibrosis

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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.

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

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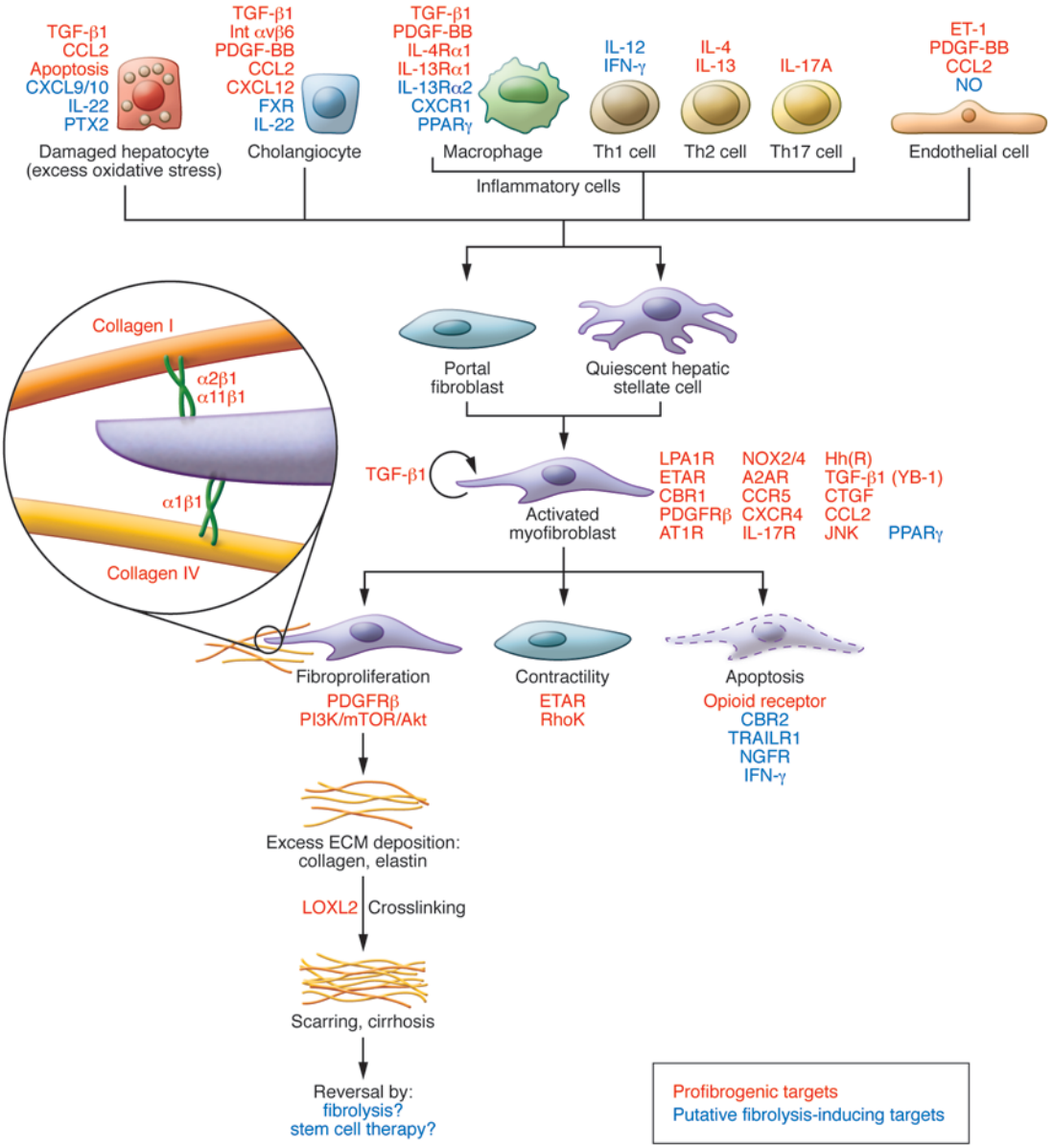

Figure 1

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-

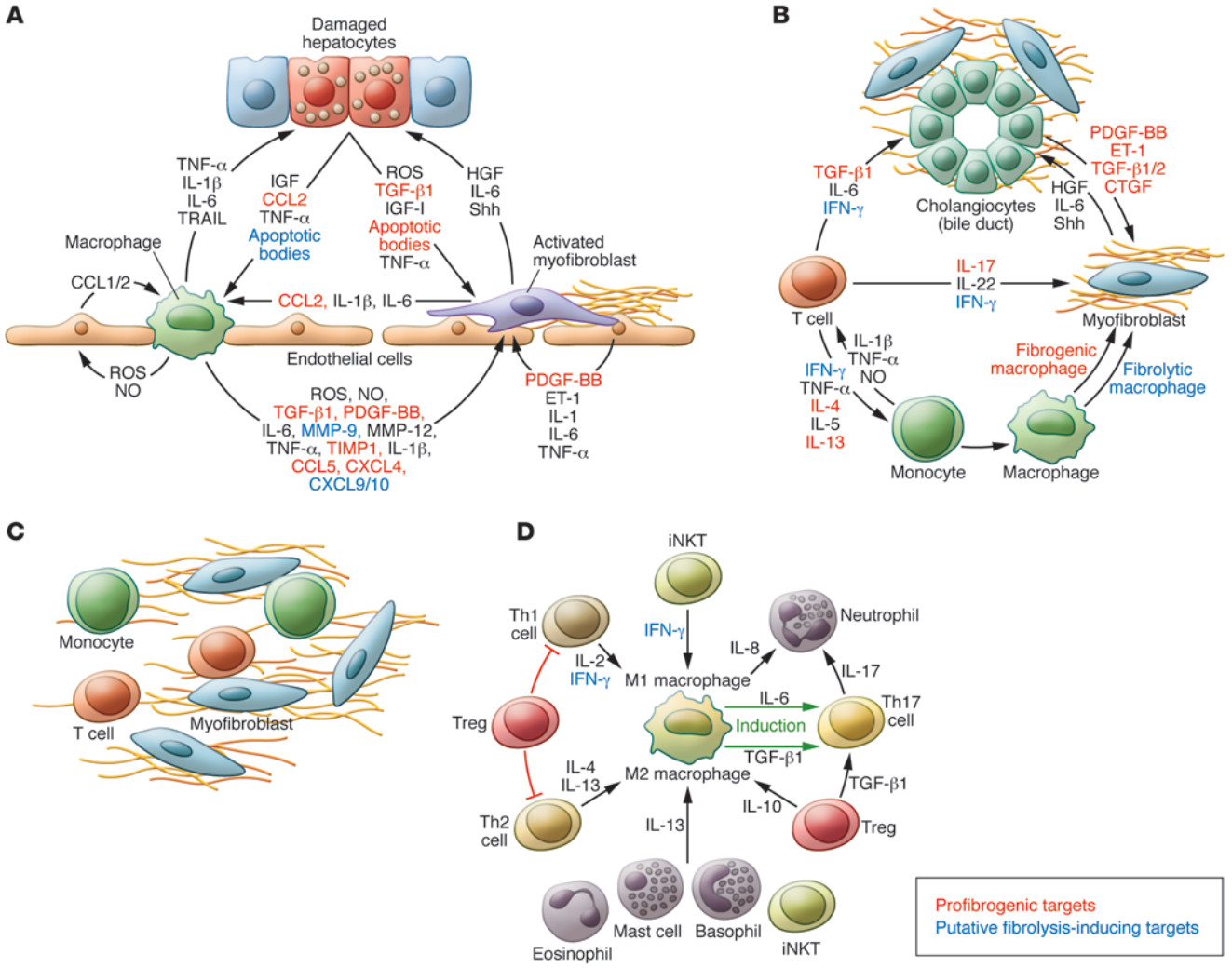

Figure 2

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

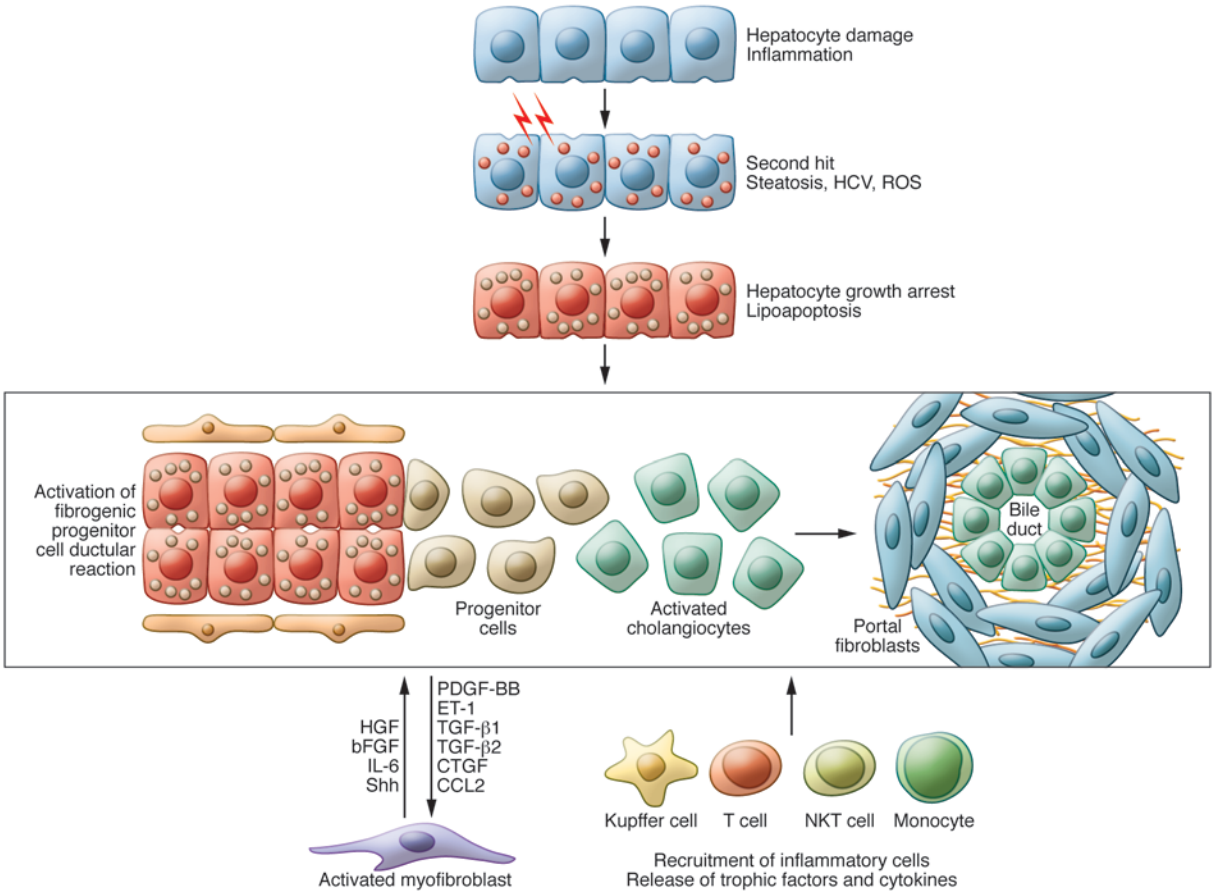

Figure 3
Activated cholangiocytes as drivers of fibrosis progression. Activated cholangiocytes are related, if not identical, to biliary progenitor cells. These cells proliferate in active biliary diseases and during massive hepatocyte growth arrest or apoptosis, as in severe NASH, ASH, or viral hepatitis. 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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