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Antifibrotic Therapies in the Liver

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Abstract

Significant progress has been made in understanding the principles underlying the development of liver fibrosis. This includes appreciating its dynamic nature, the importance of active fibrolysis in fibrosis regression, and the plasticity of cell populations endowing them with fibrogenic or fibrolytic properties. This is complemented by an increasing array of therapeutic targets with known roles in the progression or regression of fibrosis. With a key role for fibrosis in determining clinical outcomes and encouraging data from recently Food and Drug Administration-approved antifibrotics for pulmonary fibrosis, the development and validation of antifibrotic therapies has taken center stage in translational hepatology. In addition to summarizing the recent progress in antifibrotic therapies, the authors discuss some of the challenges ahead, such as achieving a better understanding of the interindividual heterogeneity of the fibrotic response, how to match interventions with the ideal patient population, and the development of better noninvasive methods to assess the dynamics of fibrogenesis and fibrolysis. Together, these advances will permit a better targeting and dose titration of individualized therapies. Finally, the authors discuss combination therapy with different antifibrotics as possibly the most potent approach for treating fibrosis in the liver.

Keywords

cirrhosis; collagen; fibroblast; fibrosis; liver; macrophage; myofibroblast; progression; regression; stellate cells; therapy

Our understanding of the mechanisms and specific components underlying the development and regression of liver fibrosis has matured toward clinical translation.[1] Specialized cell types such as activated hepatic stellate cells (HSCs) and myofibroblasts (MFs)[2,3] are central effectors of fibrogenesis (see "Origin and Function of Myofibroblasts in the Liver" by Wells and Schwabe in this issue), and other cells such as liver macrophages can promote either

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fibrogenesis or fibrolysis in a context-dependent manner (see "Resolution of Liver Fibrosis: Basic Mechanisms and Clinical Relevance" by Ramachandran, Iredale, and Fallowfield in this issue). Moreover, the underlying etiology of chronic liver damage determines both the mechanism and pattern of liver fibrosis, likely necessitating different approaches to antifibrotic therapy (see below). Instead of mere quantification of collagen and considering fibrosis as an endpoint, the dynamic processes of fibrogenesis and fibrolysis—the de novo formation and removal of connective tissue, respectively, that capture the dynamic nature of even advanced fibrosis— have taken center stage. Tissue injury is the most common stimulus for fibrogenesis, and immediately results in multiple coordinated processes aimed at initiating repair and regeneration, and at activating host defense.[4] At early stages, initiating signals (DNA, adenosine triphosphate, other nucleotides and adenosine), responding cells (macrophages, platelets, liver sinusoidal endothelial cells [LSECs]), and soluble mediators (platelet-derived growth factor [PDGF], transforming growth factor-beta [TGF-β]) induce concomitant wound-healing responses, initiating repair, regeneration, and activation of host defense. With time, cells, cytokine responses, and matrix components become more specialized, but continue to have potent interactions with each other. Inflammation can either enhance the fibrogenic signal, for example, via secretion of soluble mediators (interleukin [IL] 1-β, IL-13, IL-17, and PDGF-BB), or induce fibrolysis (interferon- [IFN-] γ or IL-12). On the other hand, chronic inflammation is often regulated and dominated by the immunosuppressive TGF-β1, which is a highly potent fibrogenic factor. These interactions make inflammatory responses an attractive target, and focused anti-inflammatory approaches are expected to reduce tissue injury and fibrogenesis, without compromising liver regeneration, which is particularly attractive in inflammatory pathologies such as alcoholic and nonalcoholic hepatitis.

The differences between individuals that determine why some repair with a scar-free liver while others proceed to cirrhosis are determined by genetic and environmental factors ("second hits"), and the quantity of these different contributing factors appear to determine the outcome. Thus, the contribution of each cellular or signaling pathway may vary between groups of individuals. However, from a therapeutic perspective the situation seems manageable because the pathways that lead to fibrogenesis or induce fibrolysis are common between individuals, and only differ quantitatively. It also stresses the necessity of a personalized approach to treatment of fibrosis, using, for example, several biomarkers that quantify key fibrogenic or fibrolytic pathways. Notably, most of the pathways found for the liver are also central pathways in the development or regression of fibrosis in other organs and vice versa.[1,5]

It is important to recognize that fibrolysis is as complex and dynamic a process as fibrogenesis and provides additional therapeutic targets. Furthermore, cellular plasticity with economy of cellular populations is a common organizing principle. This is best demonstrated for liver macrophages that are key to the development of fibrogenesis as well as fibrolysis (see review by Ramachandran et al in this issue). This makes therapies that aim to delete cell populations deemed to be fibrogenic a blunt approach, which is likely to also limit fibrolysis.

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Recognition of the full spectrum of changes associated with severe liver fibrosis is vital. In addition to quantitative and qualitative changes of the extracellular matrix (ECM), including increased ECM crosslinking and stiffness, liver fibrosis is associated with loss of hepatocytes, vascular remodeling, changes in cellular populations, and overall architectural distortion. The regenerative capacity of the liver is a great asset to all therapeutic strategies. However, therapies that aim to simply remove the ECM may not be effective against all the other pathological changes, and could even further impair liver function or increase the risk of liver cancer.

Principles of Antifibrotic Therapies

The recognition of heterogeneity in many aspects of fibrosis is a necessary step in therapeutic development. The fibrogenic pathways that are activated, and the relative amplitude of the inflammatory and fibrogenic responses vary significantly depending on the insult and its primary target cell, as exemplified by Schistosoma eggs (myeloid cells, lymphocytes), nonalcoholic steatohepatitis (NASH; hepatocytes, macrophages), or biliary obstruction (cholangiocytes). Inhibition of a single pathway upstream of the fibrogenic effector cells (HSCs and MFs) will likely generate a very different response in each of these conditions. Similarly, differences between early and advanced fibrosis will be extensive, as will be therapeutic responses. Further heterogeneity is present in the variable degree of fibrosis within the same liver. The very limited liver sampling possible by biopsy has entirely missed the variation that is present, and is now being revealed by noninvasive testing including elastography.

In view of this heterogeneity and the recent success of antiviral therapy, combination therapy for fibrosis is very attractive.[6] The simplest approach in combination therapy is to target two vital but very different pathways to reduce upstream (chronic) inflammation and downstream ECM deposition. Combination therapy is also necessary because rapid, homogeneous, and monocausal fibrosis development in animal experimental data typically reveal single targets as being central to fibrogenesis, whereas modulation of such single molecules or pathways does not prove to be highly efficient in man. From the perspective of clinical drug development, the demonstration of antifibrotic efficacy in one organ makes the agent a candidate as an antifibrotic in other organs, and also a candidate for a second drug to be added as combination therapy.

Preclinical Testing

In Vitro and In Vivo Models

In vitro models are necessary for early drug discovery to advance our understanding of the molecular pathogenesis of liver fibrosis, and for high throughput testing once a target has been identified.[7] These include culture-activated HSCs and HSC lines as well as other liver cells that are contributory to the fibrogenic or fibrolytic process. However, advanced preclinical proof of efficacy requires selected animal models, preferably mouse models that permit assessment of antifibrotic efficacy in the complex multicellular context and provide information on bioavailability, pharmacokinetics, pharmacodynamics, and toxicity. Because these models are only an approximation to the human scenario, there has been a tendency to

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omit a thorough in vivo preclinical validation before initiating larger phase 2 clinical studies. Examples are the 2-year studies of interferon-γ and the highly potent peroxisome proliferator activated receptor-γ (PPARγ) agonist Farglitazar in patients with advanced-stage hepatitis C, which yielded no effect by state-of-the-art biopsy-based fibrosis readouts.[8,9]

Animal models should reproduce the varied features of human liver fibrosis. These features include the degree and pattern of inflammation, biliary versus parenchymal damage, time course, and reversibility. Incorporation of the causative agent—hepatotropic virus, alcohol, or metabolic syndrome—is ideal, but can only be achieved for some disease or using humanized mice.[10] Although no single model will perfectly represent even a given human etiology, useful predictions as to antifibrotic efficacy appear to be possible by using combinations. Thus, mice that lack the hepatocyte phospholipid flippase Mdr2 provide a model of spontaneous biliary fibrosis progression resembling primary sclerosing cholangitis, and discontinuation of toxin-administration in advanced toxin-induced fibrosis mimics advanced human parenchymal fibrosis with little tendency to reverse.[11,12] Both models are characterized by only low-level inflammation and therefore show similarities to the target patients with advanced fibrosis of low-to-moderate inflammatory activity. Drugs that work in both models (inhibiting progression and inducing regression, respectively) may have a relatively high probability to be effective in man.

There has been significant progress in the development of rodent models of NASH. Earlier models produced components of NASH including steatosis and inflammation.[13,14] Recently, diet-based models that use high-fat diets supplemented with cholesterol and fructose have captured central features of NASH including the metabolic syndrome, steatosis, inflammation, and fibrosis.[15]

An additional limitation is that the vast majority of studies are performed in a single strain of mice (typically C57BL/6), yet there are significant differences in fibrosis susceptibility between strains. Experiments are also typically done with young (6–12-week-old) mice, whereas liver fibrosis is usually a disease of older age, with older age as a risk factor for faster fibrosis progression.

Transgenic and Gene Deletion Models

Genetic models can confirm factors and mechanisms that drive fibrogenesis or fibrolysis in vivo, for example, transgenic mice with overexpression of PDGF-B, PDGF-C, or TGFβ1.[16–18] However, these models do not reflect the multifaceted nature of human liver fibrosis, and lack chronic inflammatory liver injury, a key component in the development of fibrosis and long-term complications.[19]

Finally, in vivo models have to be done in an optimal and standardized quality, coupled with fibrosis readouts that accord to state of the art. This includes (1) group sizes of > 10 animals, (2) analysis of samples of sufficient size (5%–10% of the liver), and (3) use of complementary quantitative fibrosis and fibrolysis readouts. Notably, several past studies do not satisfy these criteria.[7]

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Precision-Cut Tissue Slices

A criticism of animal studies is their unclear transferability to the humans, which may vary with the pharmacological target. Human precision-cut tissue slices (PCTS) that can be cultured for several days are ~200-μm-thick punches of liver that partly reflect the multicellular human context.[20,21] Precision-cut tissue slices can be obtained either from normal livers (resections, spontaneous fibrogenic activation ex vivo) or from cirrhotic explants. Multiple drugs can be tested in slices prepared from a small tissue block. This technology may serve as a preclinical bridge between animal models and the patient setting. However, more studies are needed for its validation.

One major obstacle is the species difference, with significant biological differences between rodents and humans.[22] An approach to identify pathways that are important for fibrosis in humans is the concept of core pathways that are required for fibrosis in multiple organs and species.[23] Increased testing of pathways in multiple organs in rodents is relatively straightforward, and able to provide a greater degree of certainty that the pathway will be important across different species. A second important issue is the high degree of homogeneity in experimental models. The test and control populations in experimental models are homogeneous across a wide range of parameters, including, age, sex, genetic background, diet, microbiome, etc. None of these will apply to the eventual human population, and it is relevant to ask if the efficacy of a compound as an antifibrotic is maintained if there is a controlled break in homogeneity in experimental models.

The Immune Response as an Antifibrotic Target

The immune response interacts with fibrogenesis and fibrolysis at multiple points, and is an attractive candidate for therapy.[24] The healthy liver is notable for a very vigorous innate and subdued adaptive immune response.[25] Among the innate cell population, liver macrophages have been most thoroughly investigated and have key functions in fibrogenesis and fibrolysis. The well-recognized resident macrophage population of the healthy liver (Kupffer cells [KCs]) are present at birth and are self-renewing.[26] After injury, KCs initiate a fibrotic response via recruitment of additional innate immune cells, including large numbers of Ly6C^{hi} inflammatory blood monocytes[27] that quickly acquire the macrophage phenotype CD11b^{+} F4/80^{+} (Fig. 1).[28–31] These infiltrating cells have the capacity to produce a wide range of cytokines, many of which have potent proinflammatory or direct profibrotic actions on HSCs and MFs, such as TNFα, IL-1β, TGF-β1, and PDGF-BB, respectively.[32,33] They also express a range of chemokines like CCL-2, CCL-3, CCL-5, CCL-7, and CCL-8, which recruit MFs and other leukocytes.[34] Targeting some of these molecules promises to be an effective antifibrotic strategy. To take TGF-β1 as an example, several strategies to block its activity have demonstrated efficacy in rodent models of liver fibrosis. These strategies include a fully humanized anti-TGF-β1 antibody (Lerdelimumab), soluble TGF-β1 receptors, blocking peptides, and a small molecule to block downstream activin receptor-like kinase activity (SB431542; NCT 00125385, 01665391, 01262001).[35–39] Similarly, inhibition of several chemokines and their receptors demonstrated antifibrotic efficacy, including CCR5, CXCR4, and CXCR3 antagonists (NCT 00393120, 01413568).[40,41] A shared concern is that these mediators affect different cell types and are involved in many processes including angiogenesis, and cellular proliferation and differentiation; their

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inhibition may have significant off-target effects as well.[42] Some of these factors, especially chemokines, will also act differently if not in an opposite, fibrolytic way upon removal of the primary insult. Liver macrophage populations that have been vital for fibrogenesis undergo a major phenotypic switch, with enhanced production of e.g., matrix metalloproteinases (MMPs) to degrade the excess ECM and the release of proapoptotic ligands such as TRAIL, which can induce HSC and MF apoptosis.[43–45] These proresolution macrophages have a distinct phenotype (CD11b^{hi}F4/80^{in}tLY6C^{low}) and gene expression profile.[32] For established fibrosis, enabling this phenotypic switch and enhancing the number of pro-resolution macrophages is an attractive antifibrotic approach.

The relative weight of the Th1 and Th2 T cell balance is an important determinant of fibrosis for innate immune and T cells.[46,47] Thus the classically proinflammatory Th1 cytokines IFNγ and IL-12 are considered antifibrotic/fibrolytic, whereas the Th2 cytokines IL-4 and IL-13 are profibrogenic.[47,48] The Th2 cytokines may be addressable by antibody-based therapies such as a bispecific antibody targeting IL-4 and IL-13.[49] By analogy, macrophages can show a classical (M1) and an alternative (M2) polarization, which is induced by the same or similar cytokines that also induce Th1 versus Th2 polarization.[50] However, there exist several subtypes of M2 macro-phages, with some of them possibly exhibiting antifibrotic effects, complicating simple Th1/M1 vs Th2/M2 polarizing approaches using cytokine (blocking) approaches.[51,52] Therefore, skewing of this balance specifically toward Th1 (and M1) is more attractive than general inhibition of the Th2/M2 pathway, although such an approach needs to be balanced because it may enhance classical inflammation and tissue destruction.

The two related innate immune cell populations natural killer (NK) and natural killer T (NKT) cells have opposite effects. Natural killer cells have an important role in limiting fibrosis by inducing cell-cycle arrest and apoptosis of activated HSCs.[53,54] Conversely, depletion and adoptive transfer experiments suggest that NKT cells can promote fibrogenesis, but the mechanism of their profibrotic action is not well characterized.[55] More recently, type 2 innate lymphoid cells (ILC-2), which resemble Th2 T cells, have been demonstrated to be profibrogenic via secretion of IL13 and IL33, which directly activate HSCs.[56]

All antifibrotic therapies, particularly those that exert a regulatory activity, need to consider that the liver is never affected by fibrosis alone, but also by the underlying (usually inflammatory) disease. In this respect, fibrosis needs to be addressed in the context of the original disease. Antifibrotic therapies will affect many pathways. To increase efficacy and reduce side effects, therapies for specific fibrotic diseases will have to be well selected.

Regulating Platelet and Endothelial Function

Hepatic stellate cells are positioned adjacent to liver sinusoidal endothelial cells (LSECs), and the two have close functional interactions.[57] After liver injury and the initiation of fibrosis are a loss of fenestrations in LSECs, increased expression of vasoconstrictors (ET-1 and angiotensin II), and decreased activity of vasodilators, most prominently nitric oxide (NO).[58] In addition to these classic vascular changes, LSECs contribute to deposition of ECM (e.g., fibronectin and collagens I and IV), and cytokine production (e.g., TGF-β1 and

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PDGF-BB).[59] Liver sinusoidal endothelial cells can also respond to changes in sinusoidal shear stress, with enhanced production of NO.[60–62]

Therapeutic targeting of LSECs in fibrosis has focused on their predominant role in regulating the dynamic part of intrahepatic portal hypertension, which is a major cause of morbidity and mortality in cirrhosis. Interventions have included broad spectrum kinase inhibitors such as sorafenib or sunitinib, and inhibitors of vascular endothelial growth factor and endothelial growth factor. Such interventions have resulted in changes that go beyond the hemodynamic to include reduction of fibrotic matrix.[63,64] It is unclear how much of this reduction in fibrosis is due to regulation by LSECs, and how much of it is due to non-LSEC actions of these agents. However, as in inflammation, angiogenic mediators, while being profibrogenic during progression, can promote fibrolysis during regression.[65] Liver sinusoidal endothelial cells also have a key role in regulating the relative response between liver regeneration and fibrosis. This is due to a stromal factor derived pathway, which can activate the chemokine receptors CXCR7 and CXCR4.[66] After acute injury, activation of the CXCR7 pathway with recruitment of the downstream transcription factor Id1 results in a regenerative response. Chronic injury, however, results in a persistent activation of the FGF receptor 1 in LSEC that dampens the CXCR7-Id1 pathway, and activates a CXCR4 driven profibrotic pathway. Such pathways that regulate the switch between regeneration and fibrosis are excellent candidates for therapeutic intervention.[67]

Platelets are a rich source of profibrogenic factors, such as PDGF-BB and TGF-β1, but the role of platelets in fibrogenesis had been understudied.[65,68,69] Recent reports have demonstrated that most if not all PDGF-BB in liver fibrosis derives from activated platelets and that its specific inhibition with a therapeutic antibody strongly attenuates fibrogenesis. Importantly, this effect is replicated with aspirin,[8] a cheap and frequently used drug with an acceptable safety profile in early-to-moderate stages of liver disease. This finding demonstrates that we can expect marked (synergistic) antifibrotic effects by repurposing well-known drugs that are in use for other indications.

The ECM and Integrins as Antifibrotic Targets

A change in the composition and an increase in the amount of the ECM is the defining feature of all forms of fibrosis. In the normal liver, the extracellular matrix is composed predominantly of macromolecules including collagens (mainly the interstitial types I, III, V, VI, and the basement membrane types IV, XV, XVIII, and XIX), and a range of glycoproteins such as laminin isoforms and fibronectin, and several proteoglycans.[70–72] During the development of rodent and human cirrhosis, there is a 5- to 10-fold increase in the content of collagens, particularly of fibril-forming types I and III, and an increase of elastin, laminins, and proteoglycans,[73] which is accompanied by more highly crosslinked collagen fibers. The total amount of ECM is not only dependent on the rate of production, but also largely on the balance between the matrix degrading MMPs, and the inhibitors of metalloproteinases (TIMPs), especially TIMP-1.[31] The MMPs are a family of endopeptidases that are produced by a wide range of cells, and taken together can degrade all the major constituents of the ECM.[74] The TIMPs reduce MMP functionality by several mechanisms including stabilizing the proenzyme and also direct inhibition. Expression of

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TIMPs is more restricted than that of MMPs, and is high in activated HSCs. Several experiments have shown that alteration in either MMPs or TIMPs results in significant change in ECM deposition.

The ECM is not simply a downstream end product of the fibrotic cascade, but also directly feeds back onto it.[71,75] An increase in the stiffness of the fibrotic matrix initially results in HSC and MF activation via receptor- (mainly integrin) mediated signal transduction from the altered ECM to the cellular cytoplasm and back to the ECM.[76] Integrin receptors that (1) sense the collagen matrix and collagen-derived fragments, such as α1β1, α2β1, αvβ1, and αvβ3; (2) bind to fibronectin, such as αvβ3 and αvβ5; or (3) release active TGF-β1 (αvβ6 and αvβ8), which plays an important role in fibrogenesis.[71,77,78] Taken together, these integrins and other ECM receptors mediate critical interactions between the ECM and hepatic cell populations, resulting in functional changes including adhesion, migration, proliferation, differentiation, and apoptosis, as well as modulation of cytokine, chemokine, and growth factor mediated signaling.[71,79] Functional integrins are formed by noncovalent bonding of an α and a β subunit, with 24 known members in humans.[80–82] In fibrosis, interest has focused on the role of αvβ6 and αvβ8 as activators of extracellular stored latent TGFβ1, which is proteolytically processed to active TGF-β1, for example, via MMP-14 mediated cleavage, upon cellular contraction and stretching.[71,83–85] Latent TGFβ1 is tethered to αvβ6 or αvβ8 on activated cholangiocytes or HSCs/MFs, respectively via an arginine-glycine-aspartic acid motif.[77,86,87] Integrin αvβ6 is virtually absent in the healthy liver and highly expressed after a range of insults.[86,88,89] Therefore, the relative cellular specificity of the αv and especially TGF-β1 activating integrin αvβ6 permits selective inhibition of TGF-β activity in areas of mechanical stiffness and associated fibrogenesis. This is vital as total inhibition is known to result in unwanted proinflammatory changes.[90] More generally, the family of αv integrins is expressed on many liver cell populations; genetic deletion or pharmacological inhibition of all αv integrins results in attenuated fibrogenesis,[91] or in the abundant integrin αvβ3 (and αvβ5) that is mainly expressed on HSCs/ MF and macrophages.[92,93]

Collagens, the major ECM proteins in fibrosis, and elastin are stabilized via enzymatic crosslinking, which confers resistance to degradation, and thus may limit reversibility of established fibrosis.[94] There has been a focus on the family of lysyl oxidases (LOX) that crosslink fibrillary collagen mainly at the nontriple helical ends (telopeptides) of the collagen molecules.[11,95] LOX enzymes constitute a family of five members: LOX and LOX-like (LOXL) 1–4. They are secreted, copper-dependent amine oxidases with a variable N-terminal region and a conserved C-terminal domain that is necessary for catalytic activity. Expression of the LOX proteins is tightly controlled in a time- and organ-dependent manner during development, but aberrant expression and activity of these enzymes has been reported in a range of diseases associated with the ECM and in cancers,[96,97] including an upregulation of LOX and LOXL2 in Wilson's disease, primary biliary and other etiologies of cirrhosis, and in pulmonary fibrosis.[98–100] Hepatic stellate cells and portal MFs are major producers of LOX and LOXL2 in the liver.[101] A humanized antibody (Simtuzumab) that blocks LOXL2 activity is currently being assessed in a large clinical study for liver fibrosis in patients with PSC or NASH (NCT01672853, NCT01672866, NCT01672879).[99]

Targeting Fibrosis Reversal—Recent animal studies have revealed that during experimental fibrosis regression up to half of the myofibroblasts undergo senescence and apoptosis, whereas the rest acquire a quiescent phenotype.[102,103] The factors governing the inactivation of myofibroblasts are under investigation. For example, PPARγ plays a (limited) role in the re-establishment of the quiescent HSC phenotype,[102] while matrix stiffness[104] and crosslinking is currently addressed by LOXL2 inhibition (ClinicalTrials.gov, NCT01452308).[99]

Recruitment and activation of monocytes/macrophages is central to both fibrogenesis and fibrosis regression in rodents.[105] Although targeting macrophage recruitment or polarization would be an attractive approach, the functional heterogeneity of macrophage subpopulations in humans has not yet been adequately characterized. Thus no clear links can be made yet from animal studies to human disease and the macrophage subsets may be dependent on the etiology of the liver disease. One rational attempt is the use of chemokine antagonists whose role in fibrogenesis seems to be preserved among species. Therefore, preventing the early recruitment of profibrotic mononuclear cells by CCL2 inhibition intrahepatic macrophages may be shifted toward the "restorative" subset, accelerating fibrosis regression.[106]

So Many Targets: Which Ones Are Attractive for Further Clinical Development?

Fig. 2 illustrates the complexity of cellular interactions and fibrogenic or fibrolytic signals exchanged between these cells. For the past 20 years there has been a steady addition to the number of molecules and pathways that are targets for antifibrotic therapy. TGFβ1 is one of the earliest such molecules and still occupies center stage. However, systemic inhibition of TGFβ1 results in increased inflammation.[107] This spurred the targeting of specific steps in TGFβ1 activation, in a localized manner. Inhibition of integrin αvβ6, with reduction of TGFβ1 activation promises to be a highly effective and localized antifibrotic approach,[86,88,89] and clinical trials using antibodies against avβ6 are underway.[86] Connective tissue growth factor (CTGF) amplifies TGFβ1 signaling, and a monoclonal antibody targeting CTGF has shown promise in animal models of pulmonary fibrosis.[108]

Attenuating the activated phenotype of myofibroblasts is an attractive approach due to their key role in ECM deposition. Inhibition of the cannabinoid receptor 1 (CB1) reverses myofibroblast activation and attenuates experimental liver fibrosis.[109] This has passed the proof of principle state, and peripheral-acting CB1 antagonists that may circumvent adverse side effects on the central nervous system like depression are being developed.[110] In fibrotic NASH, progression is intimately linked to insulin resistance/type 2 diabetes, and the associated lipotoxic hepatocyte death and intestinal dysbiosis, providing rational targets for both anti-inflammatory and antifibrotic therapy in this condition.[111,112] Therapeutic strategies include reducing oxidative stress, improving insulin signaling, activating the farnesoid × receptor receptor (e.g., with obeticholic acid), fibrosis-targeted inhibitors of hedgehog signaling, combined peroxisome proliferator activated receptor (PPAR)α/δ agonists,[113–115] or manipulation of the altered gut microbiota using probiotics or microbiota transfer.[112,116]

Oxidative stress is an important cofactor in fibrosis, but the use of antioxidants has been disappointing.[117] This may be due to differences between animal models and human

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disease, and the fibrosis stage and cell-specific regulation of oxidant and antioxidant pathways. Activation of NADPH oxidases (NOX1, NOX 2, and NOX4) induces HSC activation[118–120] NOX4 can trigger apoptosis in hepatocytes.[120] Inhibition of NOX1/NOX4 suppresses fibrogenesis in the CCl_4 and bile duct ligation models, in pulmonary[120–122] and in interstitial kidney fibrosis. A phase II trial is underway in diabetic kidney disease (ClinicalTrials.gov NCT02010242).

Tables 1 and 2 list relevant clinical drug trials using antifibrotic agents in liver fibrosis or other organ fibrosis with fibrosis as the primary or coprimary endpoint. What is remarkable is the diversity of agents that have been tested. They range from drugs with very broad or poorly characterized mechanism (e.g., omega-3 fats and vitamin D), to specific receptor inhibitors (losartan and liraglutide), broad but fairly low intensity anti-inflammatory and antiapoptotic effects (pentoxifylline and ursodeoxycholic acid), or multikinase inhibitors (nintedanib). This is a reflection of the wide range of biological processes that are involved in the development of liver fibrosis. Due to the obvious concerns of redundant pathways, and individual heterogeneity in active pathways that lead to fibrosis, there is a significant risk that many of the single agents listed may not have significant efficacy and/or display off-target side effects. However, the past and current studies are already providing a rich resource for designing effective treatments that would also exploit drug combinations in the near future. Notably, two antifibrotics (pirfenidone and nintedanib) have recently been approved by the Food and Drug Administration and the European Medicines Agency for the treatment of pulmonary fibrosis.

The Patient Population to Be Studied

Recent reviews and guidelines highlight optimal patient selection and stratification for proof-of-concept clinical tri-als.[1,5,123] Subjects should be stratified according to the major underlying etiology, gender, signs of the metabolic syndrome, alcohol use, concomitant medications, and routine surrogates of hepatic inflammation. They should be at an intermediate stage of fibrosis (e.g., Metavir stage 2–3) for highest probability to detect dynamic changes of progression or reversal. A noninvasive measure like transient elastography or acoustic radiation force imaging is helpful for preselection before biopsy is performed, which at present is still required as entry criterion and in follow-up. The inclusion of a genetic risk score for fibrosis progression, as validated for hepatitis C virus infection, is useful, but no such score has been validated for other etiologies. Although sampling variability of biopsy is high for viral hepatitis (25–30% for a one-stage difference), and even higher for fibrosis due to NASH and biliary diseases, high-quality antifibrotic drug trials that aim at biopsies of sufficient size (at least eight portal areas) and duration (2 years), and include ~200 well-stratified patients have yielded reliable results.[8,9] Current guidelines also suggest the inclusion of several biologically plausible surrogate markers of fibrosis or fibrosis progression, such as direct or indirect serum fibrosis markers, novel imaging technologies, or measurement of portal pressure in patients with advanced fibrosis. Many of these requirements have been fulfilled, such as in the currently largest trial testing the antifibrotic effect of a Loxl2-blocking antibody (Table 1). Finally, there is much activity to develop more sensitive and specific serological markers and imaging modalities for the assessment of fibrosis and especially fibrogenesis. Once validated in ongoing studies, such

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markers and technologies could dramatically reduce the time to validate a test drug or the number of patients needed.

Combination Therapies

Considering the expected synergies of modulating two or more fibrogenic (and/or fibrolytic) pathways and the potential to decrease or eliminate the side effects that may result from targeting a single mechanism, combinations of antifibrotic (anti-inflammatory) therapies hold great promise. Agents in such combinations can address the major cause of fibrosis, such as antivirals; derive from drugs with known safety profiles that are used for other cardiovascular or inflammatory indications in a process of drug repurposing; or be specifically targeted at fibrogenic or fibrolytic cells and pathways. Such drug combinations would factor in the multifactorial etiology of fibrosis and the quantitatively divergent fibrogenic pathways in each individual, which would likely also reduce potential side effects. However, despite its promise at present there is only scant data on the efficacy of combinations of potential antifibrotic agents due to the effort needed even at the preclinical stage.[1] In man, clinical development of combination therapies that could guarantee thorough efficiency and low toxicity will only be possible with noninvasive tools that measure the effect of a given drug on its pharmacological target. In addition, we will need improved noninvasive biomarkers for the quantification of liver fibrosis, fibrogenesis, and liver function: surrogate markers for a personalized antifibrotic treatment that would permit titering of the given drugs and their combinations according to the individual antifibrotic response. The development and validation of such biomarkers has become a key focus of pharmaceutical and biotechnology companies with an interest in antifibrotic therapies.[5,7,123]

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Abbreviations

CB1	cannabinoid receptor 1
CC_{l4}	carbon tetrachloride
CTGF	connective tissue growth factor

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ECM	extracellular matrix
HSC	hepatic stellate cell
IFN	interferon
IL	interleukin
KCs	Kupffer cells
LOX	lysyl oxidase
LSEC	liver sinusoidal endothelial cell
MF	myofibroblast
MMPs	matrix metalloproteinases
NASH	nonalcoholic steatohepatitis
NK	natural killer
NKT	natural killer T
NO	nitric oxide
NOX	NADPH oxidase
PCTS	precision-cut tissue slices
PDGF	platelet-derived growth factor
PPAR	peroxisome proliferator activated receptor
TGF	transforming growth factor
UDCA	ursodeoxycholic acid

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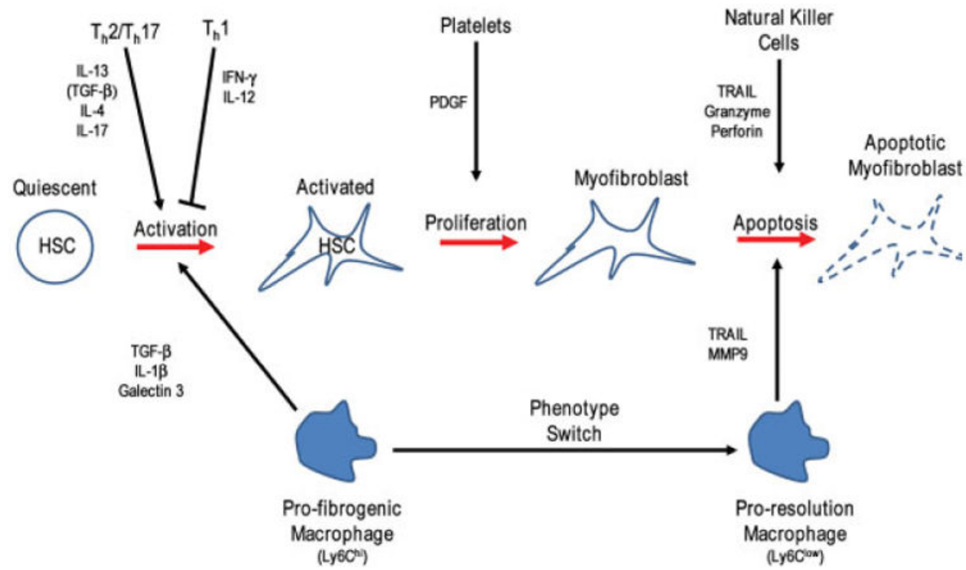

Fig. 1.

Multiple interactions between immune and profibrogenic cells. The progression of hepatic stellate cells (HSCs) from the quiescent to activated, to myofibroblasts, and eventually apoptosis is greatly influenced by paracrine signals from infiltrating blood monocytes which become tissue macrophages. At the initiation of injury, these tissue macrophages provide activation and proliferation signals, and during the resolution phase they provide apoptotic and reversion signals, but also actively digest and remove excess extracellular matrix. Additionally, innate (natural killer) and adaptive (Th1, Th2, and Th17) immune cells provide signals that can increase or decrease macrophage mediated fibrogenesis. Indirect cytokine production is shown in brackets. IL, interleukin; PDGF, platelet-derived growth factor; TGF, transforming growth factor.

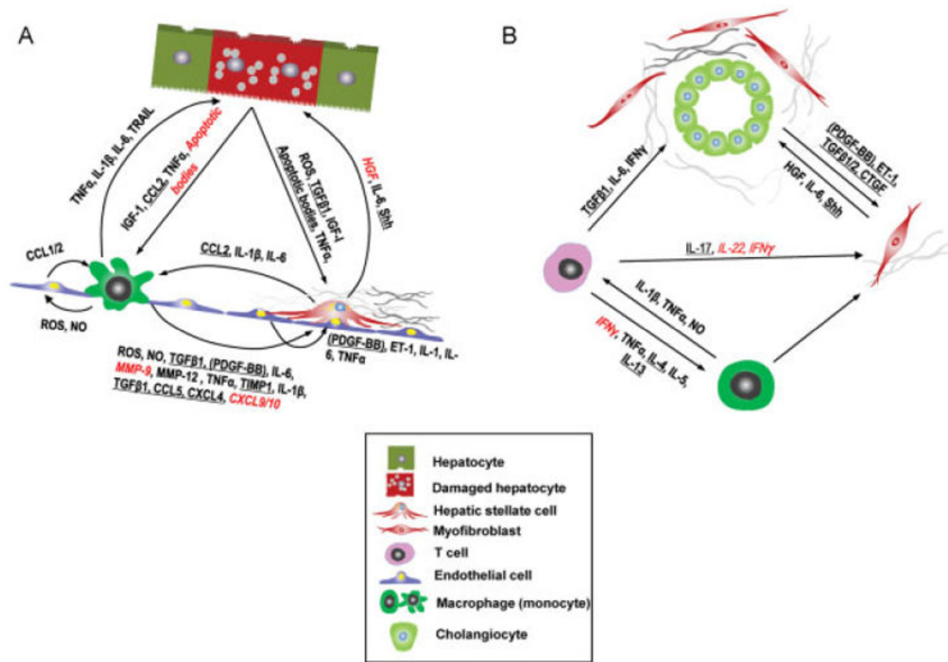

Fig. 2.

Multicellular context of fibrogenesis and fibrolysis: The postulated major cellular functional units and secreted factors that should be addressed in their complexity when designing effective antifibrotic strategies. (A) Vascular and (B) biliary unit. Profibrogenic targets are underlined, in contrast to putative fibrolysis-inducing targets in italics and red. Profibrogenic targets are underlined, in contrast to putative fibrolysis-inducing targets in italics. Modified from Schuppan and Kim.[1] Baso, basophil; CCL, CC chemokine ligand; CTGF, connective tissue growth factor; CXCL, CXC chemokine ligand; ET-1, endothelin-1; HGF, hepatocyte growth factor; IFN, interferon; IGF, insulin-like growth factor; IL, interleukin; MMP, matrix metalloproteinase; NO, nitric oxide; PDGF-BB, platelet-derived growth factor with two subunits B (in parenthesis because a recent study indicates that most if not all PDGF-BB in liver fibrosis derives from activated platelets[12]; PMN, polymorphonuclear neutrophil; ROS, reactive oxygen species; TNFα, tumor necrosis factor α; Shh, sonic hedgehog; TGFβ1, transforming growth factor β1; Th, T helper cell; TIMP, tissue inhibitor of metalloproteinases; TRAIL, TNF-related apoptosis-inducing ligand; Treg, regulatory T cell.

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Table 1

Major studies with liver fibrosis as primary or coprimary endpoint (studies with at least 50 patients)

Cause	Drug name (action), Treatment, Patients included (F,C, NR,SVR)	Efficacy	Year of completion/publication	Phase	No. of patients	NCT[Ref]
HCV (not exclusively antiviral agents)	Pentoxiphylline (anti-TNFα) vs. vit E; 1 y, r, db(F)	No results reported	2006	3	100	00119119
	IFNα-2b + R vs. IFNα-2b + R + Viusid (ascorbic acid, zinc, glycyrrhizic acid); 48 wk, r (F/NR)	Improved fibrosis score	2007	–	100	124
	Farglitazar (PPARγ agonist); 52 wk, r, db (F/NR)	No effect	2008/2010	2	225/265	00244751[9]
	GS-9450 (pan-caspase inhibitor) vs. plac; 24 wk, nr, db (F/NR)	No results reported	2010	2	307	00874796
	Irbesartan (AT1 R antagonist) vs. plac; 2 y, r, db (F/NR)	Pending	2013	3	166	00265642
	Fuzheng Huayu (Chinese herbal drug) vs. plac, 48 wk, r, db (F)	Pending	2014	2	100	00854087
	Pirfenidone (anti-inflammatory) vs. plac 2-year intervention	Pending	2014	2–3	150	02161952
HBV (not exclusively antiviral agents)	Salvianolic acid B (ingredient of Fuzheng Huayu) vs. IFNy; 6 mo, r, db (F)	No effect	2002	–	60	125
	Fuzheng Huayu) vs. plac; 6 mo, r, db (F); biopsy and serum fibrosis markers	Significant for fibrosis regression and fibrosis markers	2005		226	126
	FG-3019 (anti-CTGF mAb) vs Entecavir vs. plac; 45 wk, r, db (F)	Pending	2016	2	228	01217632
	Entecavir ± Fuzheng Huayu (Chinese herbal drug) vs. plac, 48 wk, r, db (C)	Pending	2016	4	700	02241590
HBV/HCV coinfected	Oltipraz (antiproliferative agent) vs. plac; 24 wk, r, db (F,C)	No effect	2007/2011	2	83	00956098
PBC	UDCA (hydrophilic bile acid) vs. plac; 2 y, db (F,C)	No effect	1991	3	146	127
	UDCA vs. plac; 4 y, r, db (F,C)	Lower fibrosis progression rate;	2000	4	103	128
	Obeticholic acid (FXR agonist) vs. plac; 12 mo-8 y, r, db (F); UE and serum fibrosis markers	Pending	2023	3b	350	02308111
Alcoholic hepatitis	Candesartan (ACE inhibitor); 6 mo, r, db (F)	Histological improvement; 33.3% vs 11.6% ($p = 0.020$)	2009/2012	1/2–2	85	00990639[129]

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Cause	Drug name (action), Treatment, Patients included (F,C, NR,SVR)	Efficacy	Year of completion/publication	Phase	No. of patients	NCT[Ref]
PSC	GS-6624 (anti-LOXL2 mAb) vs. plac; 96 wk, r, db(F)	Pending	2015	2	225	01672853
NASH	Orlistat (pancreatic lipases inhibitor) vs. 1400 kcal diet (30% fat); 36 wk, r, ol (F)	No results reported	2006	4	50	00160407
	Pioglitazone (PPARγ agonist) vs. plac; 6 mo, r, db	No effect	2006	4	55	00227110[130]
	Pioglitazone vs. plac; 1 y, r, db (F)	Decreased fibrosis progression	2008	–	74	131
	Pioglitazone vs. vit E vs. plac; 2 y, r, db (F)	Trend for decreased fibrosis progression for Pio groups	2009/2010	3	247	00063622[132]
	Rosiglitazone (PPARγ agonist) vs. plac; 1 and 2 V, r (F)	No effect on fibrosis	2010	-	53	133
	Pentoxifylline (anti-TNFα) vs. plac; 1 y, r, db (F)	Improved steatosis, lobular inflammation and fibrosis	2010/2011	2	55	00590161[134]
	Rosiglitazone (Rosi) vs. Rosi + Metformin vs. Rosi + Losartan; 48 wk, r, ol (F)	No effect on fibrosis	2011	-	137	135
	High-dose UDCA vs. plac, 1 y, r, db (F)	Significant reduction only of FibroTest	2011	3	126	136
	Metformin (AMP kinase activator, antidiabetic); 1 y, r, db (F)	No results reported	2012	4	80	00134303
	Metformin vs. insulin; 1 y, r, (C)	Pending	2016		126	NCT02234440
	Liraglutide (GLP-1 agonist) vs. plac; 48 wk, r, db(F)	No results reported	2013	2	52	01237119
	Pentoxifylline + vit E vs. vit E; 3 mo (biopsy), r, db(F)	No results reported	2013	3	120	01384578
	Losartan (AT1R antagonist) vs. plac; 2 y, r, db(F)	Pending	2014	3	214	01051219
	Obeticholic acid (FXR agonist) vs. plac; 72 wk, r, db(F)	Significant for steatosis, lobular inflammation; marginally significant for fibrosis	2014	2	280	01265498[137]
	Pioglitazone (PPARγ agonist) vs. vit E vs. plac; 1.5 and 3 y, r, db (F)	Pending	2014	4	90	00994682
	GS-6624 (anti-LOXL2 mAb; 75 mg vs. 125 mg) vs. plac; 100 wk, r, db (F)	Pending	2015	2	225	01672866
	GS-6624 (200 mg vs. 700 mg) vs. plac; 100 wk, r, db (F,C)	Pending	2015	2	225	01672879
	GFT505 (dual PPAR a/5 agonist); 52 wk, r, db (F)	Pending	2015	2	270	01694849

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Cause	Drug name (action), Treatment, Patients included (F,C, NR,SVR)	Efficacy	Year of completion/publication	Phase	No. of patients	NCT[Ref]
	Pioglitazone (Pio) vs. vit E vs. vit E + Pio vs. plac; 1.5 and 3 y, r, db (F)	Pending	2015	4	90	01002547
	Vit D vs. lifestyle counseling; 2 y, r, ol (F)	Pending	2014	3	200	01623024
	Vit Dvs. plac; 48 wk, r, db (F)	Pending	2015	2	60	01571063
	Omega-3 (fish oil) vs. plac; 1 y, r, db (F)	No results reported	2010	2/3	64	00681408
	Omega-3 (fish oil); 18 mo, r, sb (F)	No results reported	2013	2	100	00760513
	Docosahexaenoic acid; 2 y, r, db (F)	No results reported	2011	1/2	60	00885313
	Eicosapentaenoic acid vs. plac; 1 y, r, db (F)	No results reported	2012	2	243	01154985
	Diamel (dietary supplement) vs. plac vs. lifestyle counseling; 52 wk, r, db (F)	No results reported	2012	3	158	00820651
	PolypiII (atorvastatin, valsartan); no biopsy (UE); 5 y, r, ol (F)	No results reported	2018	3	1500	01245608
NASH Surgery	Bariatric surgery (meta-analysis of 21 cohort studies) (F,C)	Variable effect	2010	–	1643	138

Abbreviations: ACE, angiotensin-converting enzyme; ATIR, angiotensin II receptor type 1; C, cirrhosis; CTGF, connective tissue growth factor; db, double-blind; F, fibrosis; FXR, farnesoid × receptor; GLP-1, glucagon-like peptide-1; IFN, interferon; IL, interleukin; LOXL2, lysyl oxidase-like 2; mAb, monoclonal antibody; NCT, number at ClinicalTrials.gov; nr, nonrandomized; NR, nonresponders; ol, open-label; plac, placebo; r, randomized; retro, retrospective analysis; TNFα, tumor necrosis factor α; UDCA, ursodeoxycholic acid; UE, ultrasound elastography; vit, vitamin.

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Table 2

Studies in pulmonary and other fibrosis with fibrosis as primary or coprimary endpoint (studies with at least 50 patients)

Fibrosis	Drug name/Treatment	Efficacy	Year of completion/publication	Phase	No. of patients	NCT[Ref.]
Pulmonary	Etanercept (anti-TNFα) vs. plac; 48 wk, r, db	No effect	2005/2008	2	88	00063869[139]
	N-acetylcystein (NAC, antioxidant) vs. plac; 1 y, r, db	Worsening of FVC and DL_{co} in NAC-arm, no change in mortality	2005	1/2	182	140
	Bosentan (dual ET-1AR and ET-1 BR antagonist) vs. plac; 1 y, r, db Bosentan vs. plac; 12, 21 and 3 y (biopsy), r, db	Worsening of PFT; decline in FVC, DL_{co} and 0_2 saturation. No significant effect	2005/2008 2010/2011	2/3 3	158 616	00071461 00391443
	Imatinib (kinase inhibitor) vs. plac; 92 wk, r, db	No effect	2010	2/3	120	00131274[141]
	Ambrisentan (ET-1AR antagonist) vs. plac; 92 wk, r, db	Terminated due to lack of efficacy	2012	3	600	00768300[142]
	Pirfenidone (anti-TGFβ, anti-TNFα, anti-IL-1β) vs. plac, 72 wk, r, db Pirfenidone vs. plac; 52 wk, r, db Pirfenidone vs. plac; 52 wk, r, db	Study 004: reduced decline in FVC with high-dose pirfenidone	2008	3	435	00287716[143]
		Study 006: no difference in FVC Significant worsening of FVC Improved FVC, no difference in survival	2008 2010 2014	3 3 3	344 275 555	00287729[143–145]
	BIBF1120 (Nintedanib, multi-RTK inhibitor) vs. plac; 1 y, r, db BIBF1120 vs. plac; 52 wk, r, db BIBF1120; 3 y, nr, ol	Significantly reduced FVC decline and incidence of exacerbations Significantly reduced FVC decline Pending	2011 2014 2015	2 2	432 1066 198	00514683[146] 01170065
	CNT0888 (anti-MCP1/CCL2 mAb) vs. plac; 74 wk, r, db	No results reported	2012	2	126	00786201
	QAX576 (anti-IL13 mAb); 4 wk, nr, ol	No results reported	2009	2	52	00532233
	FG-3019 (anti-CTGF mAb); 109 wk, ol	Pending	2014	2	84	01262001
Myelofibrosis	GS-6624 (anti-LOXL2 mAb); 24 wk, r, ol	Pending	2014	2	54	01369498

Abbreviations: ATIR, angiotensin II receptor type 1;CTGF, connective tissue growth factor; CXCR2, CXC chemokine receptor type 2; db, double-blind; DL_{CO}, diffusing capacity of the lungs for carbon monoxide; ET-1A (B)R, endothelin-1 receptor type A(B); FVC, forced vital capacity; HMGCoAR, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; LOXL2, lysyl oxidase-like 2; mAb, monoclonal antibody; MCP1/CCL2, monocyte chemoattractant protein-1/CC chemokine ligand-2; mTOR, mammalian target of rapamycin; NCT, number at ClinicalTrials.gov; nr, nonrandomized; ol, open-label; PFT, pulmonary function test; plac, placebo; r, randomized; RTK, receptor tyrosine kinase; TNFα, tumor necrosis factor α.