Abstract: The concept that liver fibrosis and cirrhosis were being static and therefore irreversible, is outdated. Indeed, both human and animal studies have shown that fibrogenesis is a dynamic and potentially reversible process that can be modulated either by stopping its progression and/or by promoting its resolution. Therefore, the study of the molecular mechanisms involved in the pathogenesis of liver fibrosis is critical for the development of future antifibrotic therapies. The fibrogenesis process, common to all forms of liver injury, is characterized by the increased deposition of extracellular matrix components, including collagen, proteoglycans, and glycoproteins (laminin and fibronectin 2). These changes in the composition of the extracellular matrix components alterate their interaction with cell adhesion molecules, influencing the modulation of cell functions (growth, migration, and gene expression). Hepatic stellate cells and Kupffer cells (liver macrophages) are the key fibrogenic effectors. The antifibrogenic mechanism has as a starting point the activation of Ly6C high macrophages, which can differentiate into macrophages with antifibrogenic action.

The current therapeutic target in patients with liver cirrhosis should focus on the eradication of the causal agent on the development of new antifibrogenic drugs. The development of these drugs must meet three premises: patient safety; in non-cirrhotic phases, must achieve a down-staging or at least stabilization and slowing the progression to cirrhosis must be achieved, whereas in the cirrhotic stage, the objective should be to reduce fibrosis and portal pressure.

Key words: fibrosis, LOXL2, portal hypertension, hepatic stellate cells, regression cirrhosis.
INTRODUCTION

Chronic liver disease is a major cause of morbidity and mortality in developed countries. Regardless of the causal agent of the liver disease (chronic viral hepatitis B or C, alcoholic or nonalcoholic steatohepatitis), all types of liver disease have a common pathophysiological mechanism, fibrogenesis, cirrhogenesis, and the subsequent development of portal hypertension syndrome. After an acute injury, liver regeneration can be completed in a short time; however, when the noxa persists over time, a chronic wound healing response is established, leading to the replacement of parenchymal cells by extracellular matrix components (EMC) [1-3]. Although this mechanism could be initially beneficial, progressive accumulation of EMC will gradually generate disorders—abnormalities in the vascular architecture and a scarred parenchyma with a long-term outcome of hepatic dysfunction and cirrhosis.

The concept that liver fibrosis and cirrhosis were being static and therefore irreversible, is outdated. Indeed, both human and animal studies have shown that fibrogenesis is a dynamic and potentially reversible process that can be modulated either by stopping its progression and/or by promoting its resolution [4]. Therefore, the study of the molecular and cellular mechanisms involved in fibrogenesis is critical for establishing future antifibrotic therapies [4,5]. Furthermore, different patterns of fibrosis progression have been described on the basis of their etiology. In chronic viral hepatitis B and C, it is characteristic that the presence of interface hepatitis and portal-central vein bridging is characteristic in alcohol-related liver fibrosis, predominates—the deposition of EMC in the space of Disse around sinusoids (capillarization of the sinusoids) or hepatocytes accompanied with perisinusoidal or pericellular fibrosis (also in nonalcoholic fatty liver disease) predominates. Finally, biliary fibrosis is associated with the proliferation of bile ductules and periductular myofibroblasts, which leads to the formation of portal-portal fibrotic septa [5-7].

Mediators of liver injury could be different in order to yield respect to the biology of the liver disease. For example, in alcoholic liver disease (ASH) we found lipopolysaccharide (LPS)—binding protein (LBP) or Fe; in viral hepatitis, HCV/HBV proteins; in nonalcoholic steatohepatitis (NASH) glucose and adipocytokines; in primary biliary cholangitis (PBC), bile acids; and in hemochromatosis, excess of iron [5].

MECHANISM OF LIVER FIBROSIS

The liver parenchyma consists of the liver's own epithelial cells (hepatocytes), endothelial cells, and non-parenchymal cells, including hepatic stellate cells (HSCs) and Kupffer cells (KC) [2]. The hepatic microvascular functional unit is the liver sinusoid. It is formed by an endothelial lining distinguished by fenestrations of pores and is separated from the hepatocytes by the subendothelial space of Disse, where HSCs reside [6]. This space allows the metabolic exchange between blood and liver cells. The fibrogenesis process, common to all forms of liver injury, is characterized by the increased deposition of EMC components, including collagen deposition (collagen types I > III > IV), proteoglycans, and glycoproteins (laminin and fibronectin 2). These changes in the composition of the EMC lead to an alteration of their interaction with cell adhesion.
molecules, influencing the modulation of cell functions (growth, migration, and gene expression) [7]. HSC and Kupffer cells (liver macrophages) are the key elements in fibrogenesis, and the transdifferentiation to Ly6C<sup>hi</sup> macrophages constitutes the basis for the regression of fibrosis. The mechanism of hepatic fibrosis can be divided into three parts: (Figure 1)  

**Figure 1.** The mechanism of hepatic fibrosis: progression and regression steps. Legend: PDGF: platelet-derived growth factor; TGBβ: transforming growth factor β; TNF: tumor necrosis factor; IL-1β: interleukin, HSC: hepatic stellate cells; TRAIL: related apoptosis-inducing ligand receptor; MMP: metalloproteinase; ECM: extracellular matrix.  

1. Acute liver damage triggers the activation of Kupffer cells which coordinate the regenerative response; however, if a chronic damage persists, over-activation of these cells results in the release of proinflammatory cytokines (CCL2 and CCL5) and a stimulation of the bone marrow for the generation of activated Ly6C<sup>hi</sup> macrophages [8,9].  

2. This inflammatory magma induces the transdifferentiation of the HSCs to activated HSC or myofibroblasts that produce substances of the extracellular matrix (collagen type I, III, and IV; fibronectin; laminin; and proteoglycans) [10].  

3. At this point, it is crucial the role of tissue hypoxia leading to the formation of new blood capillaries is crucial. This neangiogenesis, is the beginning of the generation of portosystemic collateral vessels and portal hypertension syndrome and can lead to the so-called "point of no return" [11,12].  

In order to understand the potential treatment options for liver fibrosis, we summarize the fibrogenic effectors involved in liver fibrosis (Figure 2).
**Figure 2.** Effectors in liver fibrosis and potential therapeutic goals. **Legend:** CXCR: chemokine receptor; PPARγ: peroxisome proliferator-activated receptors; CCL: Chemokine ligand; CBR: cannabinoid receptor 1; ET-1: endothelin-1; ETAR: endothelin A receptor; FXR: farnesoid X receptor; Hh(R): hedgehog (receptor); Int: integrin; LPA1R: lyso-phosphatidic acid receptor 1; NGFR: nerve growth factor receptor; PTX2: pentraxin 2; TRAIL R: TRAIL receptor; CBR1: carbonyl reductase 1; HGF: hepatocyte growth factor; NGFR: nerve growth factor receptor.

**Damaged hepatocytes.** Hepatocyte apoptosis is a strong inducer of fibrogenesis (chiefly in liver diseases with strong oxidative stress, such as ASH and NASH). Phagocytosis of apoptotic hepatocytes by myofibroblasts triggers their fibrogenic activation via NADPH oxidase 2 (NOX2) and the JAK/STAT and PI3K/Akt pathways. [13]

**Activated myofibroblasts** derive from both activated hepatic stellate cells and portal fibroblasts, which are the principal producers of scar, but are also involved in fibrosis regression through the release of ECM-degrading proteases [14,15]. Furthermore, several vascular mediators promote HSC contractility, like endothelins (produced by endothelial cells), and nitric oxide (NO),...
Biliary progenitors. Biliary progenitor cells (activated cholangiocytes) secrete several factors are involved in the attraction and activation of HSCs to myofibroblasts to proliferate and deposit ECM. They are more resistant to oxidative stress and hepatocyte death.[13].

Liver sinusoidal endothelial cells (LSECs). Hepatic (neo-)vascularization with LSEC activation and proliferation is tightly associated with perisinusoidal fibrosis (capillarization of the sinusoids) because LSECs contribute to ECM production, and secrete both cytokines (e.g., TGF-1 and PDGF-BB), that which activate HSC, and vasoconstriction factors (e.g., endothelin-1).[17.

T cells. Regulatory T cells appear to either favor or inhibit fibrogenesis. CD4+ T cells with a Th2 polarization, promote fibrogenesis due to the production of IL-4 and IL-13, whereas CD4+ Th1 cells have an antifibrotic effect [[18.]] Th17 cells are clear drivers of fibrosis in multiple tissues and secrete IL-17A, which drives fibrogenesis directly in terms of myofibroblasts and indirectly via the stimulation of TGF-1 release from inflammatory cells.[19-21].

Monocytes. Monocytes are essential in inflammation and fibrosis, because they are precursors of fibrocytes, macrophages, and dendritic cells. Activated monocytes are also involved in adaptive immune responses (proinflammatory monocytes CD14+CD16+) promoting fibrogenesis. CCL2 and its receptor CCR2 promotes monocyte recruitment in the inflammatory lesion, and CXCL9 (and as well as CXCL10) prevents pathological angiogenesis and fibrogenesis via the activation of their receptor, CXCR2.[22].

Macrophages. Macrophages have a double function: they are fibrogenic during fibrosis progression and fibrolytic during its reversal. The antifibrogenic mechanism has as a starting point the activation of Ly6C[low] macrophages, which can differentiate into macrophages with antifibrogenic action (inactive macrophage Ly6C[high]) [6,23].

As noted, recent evidence coming from human studies and animal models has overtaken the old dogma of the irreversibility of liver fibrosis irreversibility, and it is now considered a dynamic and reversible process [7]. Fibrosis reversibility has as starting points. The activation of Ly6C[high] macrophages, which can differentiate into macrophages with fibrolytic action, or inactivation of a macrophage Ly6C[low] if the noxa is removed (e.g., the eradication of hepatitis C) [25]. The CXCR1 receptor seems to be involved in such differentiation, and its overexpression is associated with the phenotypic transformation into Ly6C[low] macrophages. These macrophages release large amounts of extracellular matrix metalloproteinases (MMP-9 and MMP-13) and the anti-inflammatory cytokine IL-10, which are involved in the resolution of fibrosis. The Ly6C[low] macrophages lose their ability to stimulate and maintain the myofibroblast phenotype. As a result, activated stellate cells die from apoptosis (programmed cell death) or revert to quiescent cells (senescence) [26]. These concepts are summarized in Figure 22.

These data suggest that the activation of apoptosis could be a good therapeutic target of fibrosis reversibility. This approximation, however, faces the problem of the induction of fibrogenesis through apoptotic cells. Interestingly, inactivated HSCs that persist in the liver after an acute injury are much more sensitive to a new transdifferentiation to myofibroblasts. Thus, in a previously damaged liver, a new insulin injury will activate faster the profibrogenic transformation faster.[26].
At this point, we must raise three questions arise: Can fibrosis be completely reversed into a healthy liver? Does the potential liver regeneration depend on the baseline stage of fibrosis? Is causal treatment of the liver disease enough in the early stages but not at later stages?

To date, the etiological treatment of chronic liver disease has been the basis of research in hepatology, with great success in both inhibiting the replication of hepatitis B and eradicating hepatitis C, even advanced stages of the disease with the appearance of new direct antiviral drugs. These strategies have been successful in blocking liver injury and therefore the progression to advanced fibrosis, and even achieving its reversion of advanced fibrosis. In a multicenter randomized placebo-controlled trial involving 651 patients with HBV cirrhosis [27], lamivudine decreased the risk of severe liver complications (7.8% vs. 17.7%; p=0.001). Similar findings have been reported in patients with advanced non-cirrhotic HCV disease, in whom the risk of liver cancer, liver failure, and all-cause and liver-related mortality decreased substantially during a ten-year follow-up in patients achieving sustained viral response (SVR) [28].

A recent study has evaluated in 304 patients with SVR, the regression of fibrosis in 304 patients with SVR through liver stiffness measurement (LSM) using FibroScan®. In comparison to its baseline values, LSM was unchanged in 60%, decreased in 41.5%, and worsened in 7.1% of the patients. Among the 130 patients classified as F4, 78 remained stable, 22 went to F3, 11 to F2, and 19 to F0–F1 [29].

As far as the influence of viral clearance on portal hypertension is concerned, our group has recently shown that SVR was associated with a normalization of the hepatic venous pressure gradient (HVPG) in more than 50% of HCV patients with previously portal hypertension (HVPG > 6 mmHg) and with a significant reduction in the FibroScan® values (<7.1kPa) in a third of the patients [28]. The regression of hyperdynamic circulation, activation of vasoactive systems, and involution of portosystemic collateral networks, are aspects that is currently under evaluation, [30]. In a recent Spanish study with 226 cirrhotic patients (21% Child B and 75% with esophageal varices), it was demonstrated that despite the fact that the HVPG decreased from 15mmHg to 13mmHg at 24 weeks after treatment with direct antiviral agents (DAAs), clinically significant portal hypertension (HVPG>10 mmHg), persisted in 78% of the cases [33].

Another issue to be taken into account in the method is the validity of the elastographic (mainly Fibroscan®) and other serological methods in the follow-up of post-SVR fibrosis and steatosis. [31,32]. Fibroscan® improves the detection of an after viral elimination due to the fall of necroinflammatory activity, but we know it is not a good screening method for clinically significant portal hypertension since it was recently shown that at 24 and 96 weeks after treatment with DAAs, 43% and 28% of patients, respectively, with a Fibroscan® of <13.6kPa have a HVPG of >10 mmHg [33].

Although obviously patient prognosis obviously improves with viral suppression, the risk of hepatocarcinoma (HCC) development still exists in those patients with no regression of advanced fibrosis. The development of new antifibrogenic drugs is most needed in these patients [29–34]. In Figure 5, we summarize the potential therapeutic targets are summarized.
Recently, the research of biochemical changes affecting fibrosis irreversibility has identified lysyl oxidase-like 2 (LOXL-2), an enzyme that promotes the network of collagen fibers of the extracellular matrix [35]. The lysyl oxidase gene family is currently composed of five variants (LOX and four LOX-like variants, LOXL1–4). The protein isoforms are synthesized as inactive proenzyme and secreted into the extracellular environment where they are cleaved to a mature and functional form.

LOX and LOXL-4 proteins share a highly conserved C-terminal region that contains the catalytic domain, while the N-termini are less conserved among the five members and are supposed to determine their functional role and tissue distribution [36].

LOX is secreted as proproteins (proLOX) which are proteolytically cleaved to release the free catalysts and the N-terminal propeptide regions which immediately precede the catalytic domains. ProLOX molecules are catalytically quiescent but is activated by proteolytic cleavage between Gly162 and Asp163 (rat LOX sequence) by procollagen C-proteinase. The redundancy of proteases involved in the maturation and activation of LOX underscores the importance of LOX activity in ECM homeostasis [33]. LOXL is a Cu-dependent amine oxidase capable of a post-transcriptionally modification of type 1 collagen (the main collagen in hepatic fibrosis) and elastin, by oxidation of peptidyl lysine and hydroxyllysine residue collagen, transforming it to allysine, responsible for the formation of crosslinks that stabilizes collagen and elastin in the extracellular matrix.

LOXL2 (animal and human) was detected in biliary fibrosis and NASH liver cirrhotic models [35–37].

Regarding oncogenesis, chronic liver disease is a well-known risk factor for HCC [28]. One of the mechanisms by which this chronic inflammation leads to fibrosis, cirrhosis, dysplastic nodules, early HCC, and the end to advanced metastatic HCC, is the induction of TGF-β, Smad4, and LOXL2. Activation of this pathway has been shown in the progression of breast cancer and the appearance of intrahepatic metastasis of HCC. It has been recently published in Hepatology [38].

The involvement of LOXL2 in the transformation of the extracellular matrix in a “prooncogenic” tissue that favours the metastatic niche of intrahepatic HCC has been recently published in Hepatology [38]. LOXL2 inhibition can decrease cell numbers, proliferation, colony formations, and cell growth, and it can induce cell cycle arrest and increase apoptosis. Expression levels of LOXL2 were markedly increased in matched adjacent non-tumor tissue compared with levels in tumor tissue samples, and this difference gradually increased with higher histological grade and more advanced hepatocellular tumors [39]. Hypoxia increases LOXL mRNA, LOXL protein, and secreted LOXL activity, resulting in enhanced invasive migration required for metastatic spread. Although LOXL is known to be induced and/or activated by growth factors such as TGF-β, hypoxia might be more clinically relevant with regard to tumour progression [39].

While the relationship of serum LOXL2 with the stage and progression of pulmonary fibrosis has been demonstrated [40], its relationship with liver fibrosis is still under evaluation. In the Liver Meeting 2015, three poster communications did not demonstrate that a correlation between changes of serum LOXL2 and changes of non-invasive markers of fibrosis or HVPG after SVR were not correlated. However, a longer follow-up is needed to fully evaluate this correlation [41–43]. Our own group had recently communicated at the 2019 Annual Meeting of Spanish Association for the
Study of the Liver that LOXL2 serum levels decreased after viral eradication, in a cohort of 271 patients with HCV liver disease, two years post RVS with DAAs—that LOXL2 serum levels decrease after viral eradication. However, we found a high variability of values among the patients (depending on the presence of inflammatory comorbidities, such as inflammatory diseases) and in a small cohort of pretreatment cirrhotic patients, we observed a higher tissue expression in most of patients, and this was related to serum expression but not with liver fibrosis stage posttreatment [44].

In 2010, an interesting paper published in Nature Medicine [45], studied the role of LOXL2 in rodent pulmonary/liver fibrosis and cancer rodent models and the benefit of inhibition by a monoclonal antibody (AB0023 and the humanized variant AB0024). AB0023 inhibited vessel branching, number, and length in a dose-dependent manner, with complete inhibition at the highest concentration. Treatment with monoclonal antibody (AB0023) improved mouse survival at 25 days (100% in the treated group vs 50% in the control group, p < 0.006) and reversed fibrosis F3 to F1 (assessed according to the METAVIR score) in most models. Likewise, a decrease was observed in the expression levels of αactin in myofibroblasts which activates both fibrogenesis and oncogenesis.

Given the above findings, a humanized IgG4 monoclonal antibody against LOXL2, SIMTUZUMAB® (Gilead Sciences SA) has been developed. SIMTUZUMAB® has already been studied in idiopathic pulmonary fibrosis and colorectal and pancreatic cancer [47-49]. Accordingly, a recent randomized, double-blind, controlled, phase 2 trial has evaluated its efficacy in more than 500 patients with idiopathic pulmonary fibrosis in more than 500 patients. The preliminary results did not support the use of SIMTUZUMAB® for patients with this disease, because it did not improve progression-free survival [40,47]. Finding the experience form colorectal and pancreatic cancer did not support a clear clinical benefit [48-49].

To date, the experience tests in patients with liver fibrosis treated with SIMTUZUMAB® did not improve show improvements of clinical and fibrosis outcomes. The first report was communicated at their EASL meeting, Amsterdam, 2013. Twenty patients with liver fibrosis (F1 to F3 in METAVIR) [50] received three intravenous injections of SIMTUZUMAB® divided in two groups with 10 mg/Kg or 20 mg/Kg, respectively over the course of six weeks. In both groups, an improvement in liver function test was observed without serious side effects related to the treatment (abdominal pain, headache, muscle pain, or fatigue). The results in the regression of liver fibrosis have not been published yet. Recently, it has been published three trials in NASH and primary sclerosing cholangitis (PSC) have been published. SIMTUZUMAB® is ineffective in decreasing hepatic collagen content or hepatic venous pressure gradient for patients with non-significant fibrosis, bridging fibrosis, or compensated cirrhosis caused by NASH in association or not with ASK 1 inhibitor (selonsertib) [51-52]. In PSC, SIMTUZUMAB® or placebo treatment for 96 weeks did not lead to any significant reductions in Ishak fibrosis stage, progression to cirrhosis, or frequency of clinical events, regardless of the dose (injections of 75 mg or 125 mg) [53]. The use of SIMTUZUMAB® seems to be safe, without any reports of serious adverse effects reported.

The failure of SIMTUZUMAB® in these three trials, may be due to a number of factors: there is not a good correlation between LOXL-2 serum levels and tissue expression, and this fact could be a reason
for a non-response to the treatment. Second, the activity of Simtuzumab may be insufficient to inhibit collagen cross-linkage, and third, LOXL2 inhibition may be ineffective due to redundancy in other pathways that mediate collagen cross-linkage.

So hence, to date, the results do not support the use of monoclonal antibody against LOXL2, for the treatment of liver fibrosis. As it is shown in green in Figure 2, there are many potential therapies for liver fibrosis. The use of ASK 1 inhibitor (selonsertib) [52] or a pan-caspase inhibitor such as emricasan [54] could be a potential therapeutic option for liver fibrosis and portal hypertension. However, the huge complexity of the fibrogenic mechanism and the fact that the mediator of liver regeneration varies between patients difficult complicates the development of an effective drug—or perhaps, we need a combinations of them[55].

NON-INVASIVE EVALUATION OF LIVER FIBROSIS

Another key aspect is the assessment method of assessment of liver fibrosis. Liver biopsy remains as the gold standard for the grading of hepatic fibrosis. However, it is not a perfect method, as it is invasive and may be subject to sampling errors. That is why the development and validation of non-invasive methods (e.g., elastographic and serological) to evaluate the regression of liver fibrosis is essential [56-60]. As we described above, LSM is not a goof method for screening significant liver fibrosis post RVS [31]. The number of non-invasive methods has increased in recent years: transient, vibration-controlled shear wave and magnetic resonance elastography [59,60], acoustic force radiation impulse, magnetic resonance techniques to determine the inflammation and fibrosis score [60] or the quantification of ECM molecules [61], or dynamic markers of collagen synthesis. The data suggest that these tests have a high sensitivity and specificity for the detection of advanced fibrosis and cirrhosis, but are not highly sensitive or specific for less advanced stages of fibrosis. Perhaps, more interesting could be the approach of using quantitative liver function tests to predict the risk for future clinical outcomes, such as cholate clearances (validated in HCV patients), metabolic tests, or SPECT [Liver-spleen scan, to predict the risk for future clinical outcomes is more interesting, but more studies are needed for their implementation in the daily clinical practice [62-63].

CONCLUSION

The current therapeutic target in patients with liver cirrhosis should be focus (after eradication of the causal agent) on the development of new antifibrogenic drugs. The development of these drugs must meet three premises: patient safety; in non-cirrhotic phases, must achieve a down-staging or at least stabilization and slowing the progression to cirrhosis must be achieved, whereas in the cirrhotic stage, the objective should be to reduce fibrosis and portal pressure [7,40]. LOXL-2 inhibition by Simtuzumab does not seem to be effective in liver fibrosis.

Abbreviations:

HSC, hepatic stellate cell; NASH, nonalcoholic steatohepatitis; ASH, alcoholic steatohepatitis; PBC, primary biliary cirrhosis; KC, Kupffer cells; extracellular matrix; EM, extracellular matrix; EMC, extracellular matrix components; CXCR4, chemokine receptor; CX3CR1, fractalkine receptor; PPARγ, peroxisome proliferator-activated receptors; CCL-5, chemokine ligand; MMP, metalloproteinases; SVR, sustained virological response.
REFERENCES

40. Chien et al. Serum lysyl oxidase-like 2 levels and idiopathic pulmonary fibrosis disease progression, Eur Respir J. 2014 May;43(5):1430-4
41. Bosch et al. Correlation between noninvasive markers of fibrosis and the hepatic venous pressure gradient (HVPG) in patients with compensated cirrhosis due to nonalcoholic steatohepatitis (NASH). Hepatology 2015, Oct 2015;62 (S1), 121A
42. Afidha et al Serum lysyl oxidase-like-2 (sLOXL2) is correlated with the hepatic venous pressure gradient (HVPG) in patients with cirrhosis due to hepatitis C. Hepatology 2015, Oct 2015;62 (S1), 121A
43. Bourliere et al. Changes in liver stiffness by transient elastography (TE) and serum lysyl oxidase-like-2 (sLOXL2) in patients with cirrhosis treated with ledipasvir/sofosbuvir (LDV/SOF)-based therapy. Hepatology 2015, Oct 2015;62 (S1), 123A

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