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REVIEW ARTICLE

Reversal of NASH fibrosis with pharmacotherapy

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Abstract
NAFLD is a spectrum of liver disease starting with fatty liver at one end of the spectrum and cirrhosis or liver cancer at the other end. Worldwide, NAFLD has become one of the most common liver diseases and it has also become one of the leading indications for liver transplantation. Our understanding of the NAFLD epidemiology, pathogenesis and its progression to cirrhosis has improved over the last 2 decades. Currently, however, there are no FDA-approved treatment options for fibrosis resulting from NAFLD. A number of compounds targeting multiple pathways involved in the progression of NAFLD are currently in phase 2–3 trials. In this review, we will briefly discuss the epidemiology, the pathogenesis and the current status of treatment of NAFLD.

Keywords NAFLD · NASH · Antifibrotic agents

Introduction

Non-alcoholic fatty liver disease (NAFLD) which has a strong association with metabolic syndrome and insulin resistance is a leading cause of liver disease. According to a recent meta-analysis, the global prevalence of NAFLD is 25.24% with the highest prevalence in South America and the Middle East [1]. The prevalence in North America and Asia is estimated to be around 24% and 27%, respectively. NAFLD is a spectrum of liver disease starting with fatty liver (non-alcoholic fatty liver or NAFL) at one end of the spectrum and cirrhosis at the other end. A subset of patients with NAFLD will develop progressive liver disease histologically characterized by hepatic steatosis, inflammation and hepatocyte injury (ballooning) with or without fibrosis known as non-alcoholic steatohepatitis or NASH. Unlike NAFL, approximately 25% of NASH patients at the time of diagnosis may have advanced liver fibrosis, defined as fibrosis stage 2 (F2) or higher. There is circumstantial evidence that links the degree of fibrosis to mortality in this population [2, 3]. The presence of inflammation on initial biopsy is considered as one of the strongest predictors for progression of NASH into advanced fibrosis [4]. In the recent era, with a better understanding of the epidemiology and the complex pathogenesis of fibrosis occurring at the cellular and molecular levels, there is increasing interest in pharmacologic agents that can either reverse and/or slow down the progression of fibrosis. This review will focus on the emerging treatment options for NASH with an emphasis on antifibrotic agents.

Implications of fibrosis on all-cause and liver-related mortality in NASH

Advanced fibrosis of liver (stage 3–4) is associated with an increased all-cause mortality and liver-related mortality [5–7]. Complications of cardiovascular disease are the leading cause of death in patients with NASH followed by cancer and cirrhosis. A meta-analysis of 5 studies which included 1495 patients suggested that even those with less advanced fibrosis (stage 1) have a higher all-cause mortality and moreover, the risk of liver-related mortality increases on an exponential scale rather than a linear scale with increase in the stage of fibrosis [6]. While stage 1 fibrosis is associated with a liver-related mortality rate (MRR) of 1.4, stage 4 had a MRR of 42.30. Another study from Sweden analyzed 229 patients with NAFLD with a mean follow-up of 26.4 years and reported an increased overall mortality in

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stage 3 and stage 4 fibrosis irrespective of the NAFLD activity score (NAS) [7].

Progression of fibrosis

There are only limited prospective data on progression of fibrosis in those with NAFLD. A study in England followed 108 patients (81 with NASH and 27 with NAFL) using paired liver biopsies over a median period of 6.6 years and reported an overall progression of fibrosis in 42%, regression in 18% and stable fibrosis in 40% [8]. Out of the 45 patients who had disease progression, 26 progressed by 1 stage, 15 by 2 stages and 4 by 3 stages with a mean rate of fibrosis progression of 0.29 ± 0.24 stages/year. It is pertinent to note that 37% of patients with NAFL at index liver biopsy developed fibrosis and of these, 22% developed advanced stage 3 fibrosis.

In another longitudinal study of 52 patients (13 NAFL, 22 borderline NASH and 17 definite NASH), [9], the follow-up liver biopsy at 3 years revealed progression to borderline NASH in 5 (39%) and development of NASH in 3 (23%) in the 13 patients with only NAFL. Of those patients with NASH, 58% of patients who had NAFLD activity score (NAS) < 3 at baseline had increased NAS scores at 3 years implying that a sub-population with NAFL and NASH will progress with time. A recent meta-analysis of paired biopsies which included 11 studies consisting of 411 patients with NAFLD also corroborated the progressive nature of the disease. This study examined 150 patients with NAFL and 261 with NASH over 2145.5 person-years of follow-up and reported an overall fibrosis progression (increase by at least one fibrosis stage as compared to index biopsy) by 33.6% for the entire cohort. Patients with NAFL had an average progression by 1 stage over 14.3 years, while this was much faster for NASH (7.1 years). Furthermore, this study identified a distinct subset of patients referred to as rapid progressors (progression from baseline stage 0 fibrosis to advanced stage 3 or 4 fibrosis). Out of 52 patients with NAFLD who had stage 0 fibrosis on index biopsy, 21.2% had rapidly progressed to bridging fibrosis or cirrhosis on follow-up biopsy. It is possible that the sampling error could partially explain some of these findings.

The data from seven randomized placebo-controlled treatment trials in patients with NASH were examined recently for fibrosis progression and the results were presented in an abstract form recently [10]. This post hoc analysis showed that fibrosis progression rate was only 0.05 per year in the placebo-treated group which is significantly lower than observation cohort studies. It is important to note that treatment trials in NASH have shown a significant placebo benefit which could be related to life style modification.

Prediction models on NAFLD epidemiology and progression

Modeling studies suggest that the burden of NAFLD and NASH is projected to increase globally. Using a mathematical model, one study projected an 18.3–29.3% increase in the number of NAFLD cases in different parts of the world [11]. The greatest overall increase in the prevalence is expected to occur in China where NAFLD is estimated to increase from 246.3 million cases in 2016 to 314.5 million by 2030 corresponding to a 29.1% increase. This model also predicted a 56% increase in the number of NASH cases in the United States by 2030, and more strikingly an exponential increase of advanced fibrosis (stage F3/F4) by 124% from 3.55 million in 2016 to 7.95 million in 2030. The prevalence of decompensated cirrhosis and HCC related to NAFLD is also expected to increase globally according to this model. By 2030, the prevalence of HCC is projected to increase in China, France and United States by 86%, 125% and 130%, respectively.

Pathogenesis

The pathogenesis of NAFLD is complex and multifactorial involving genetic, epigenetic and environmental factors (Fig. 1). The traditional two-hit concept regarding the evolution of NASH has now been replaced by the multiple hit hypothesis [12, 13]. According to the traditional model, steatosis (NAFL) occurs in the liver secondary to obesity, insulin resistance and sedentary life style which represents the first hit, and the steatosis then sensitizes the liver to the second hit, which is oxidative stress and mitochondrial dysfunction triggering an inflammatory cascade resulting in steatohepatitis (NASH) and fibrosis. The multiple hit theory speculates that NAFLD and its progression are secondary to an interplay between a wide range of insults ('multiple hits') in a genetically predisposed subject. The insults may include insulin resistance (leading to de novo lipogenesis), lipotoxicity from free fatty acids, endotoxins from gut microbiomes, and genetic factors such as *PNPLA3* polymorphism [13].

In those with NASH, as in all liver diseases, liver fibrosis results from dysregulated wound healing which is characterized by excessive deposition of extracellular matrix (ECM) [14]. Although a detailed discussion on the pathogenesis of fibrosis is beyond the scope of this review, a brief summary is essential to understand the potential therapeutic interventions. Usually after an acute hepatic insult, the parenchymal cells (hepatocytes) undergo regeneration and replace the necrotic cells, a process associated with

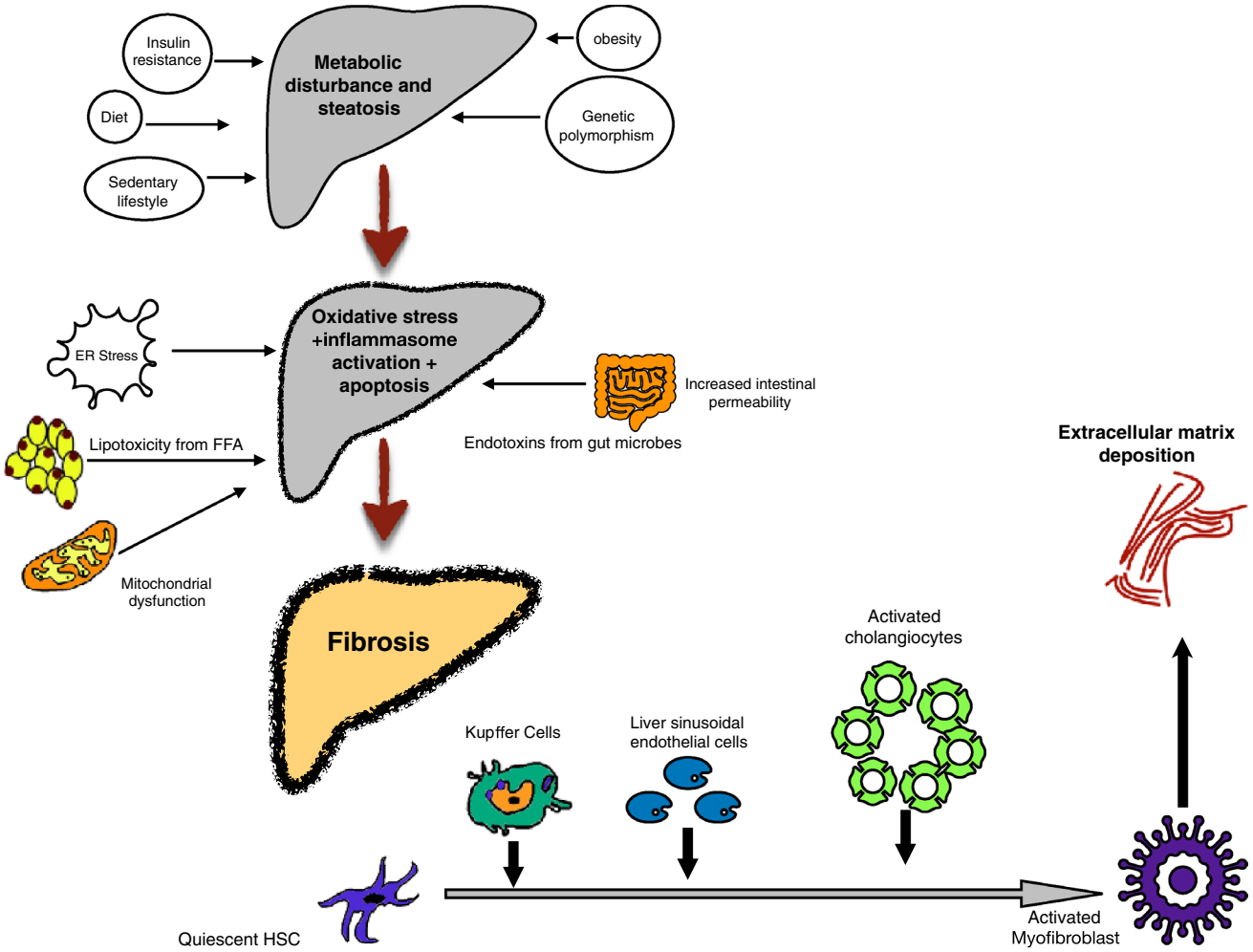

Fig. 1 Pathogenesis and progression of non-alcoholic fatty liver disease

minimal ECM formation. However, when injury is repetitive, regeneration becomes futile as regeneration is unable to keep pace with the rate of hepatocyte death and this leads to ECM deposition. Hepatic stellate cells (HS) which constitute 10% of the total liver cells play a critical role in this process by differentiating into myofibroblasts which is the main source of ECM [15]. Accumulation of free fatty acids (FFA) in hepatocytes trigger lipoapoptosis and this may be one of the mechanisms for the activation of stellate cells into myofibroblasts causing them to produce matrix proteins at a rate faster than it can be degraded. Several fibrogenic growth factors (TGF-B, PDGF, leptin) and signal pathways (Wnt, Notch, Hedgehog) are involved in this process [16]. One of the best studied pathways that has recently emerged as a promising target for novel pharmacological agents is the Hedgehog (Hh) pathway, which is briefly described in this review.

The Hedgehog gene was first discovered in 1980 by Eric F. Wieschaus and Christiane Nusslein-Volhard in *Drosophila*, and since then three mammalian Hedgehog genes have been identified: Sonic Hedgehog (Shh), Indian Hedgehog (Ihh) and Desert Hedgehog (Dhh) [17]. The hedgehog (Hh) ligand is hardly expressed in a healthy adult liver, however, lipotoxic (ballooned) hepatocytes seen in NASH release the ligands which are responsible for orchestrating a dysregulated wound healing process by activating macrophages, natural killer (NK) cells, progenitor cells, stellate cells and sinusoidal endothelial cells [18]. Studies in animal models have demonstrated that quiescent hepatic stellate cells express Hhip, a competitive antagonist of the Hh pathway accountable for keeping fibrosis at check, but after intraperitoneal injection of CCl4 (to induce cirrhosis in rat models), there is a downregulation of Hhip and increased expression of Sonic Hedgehog (Shh) ligand causing transition of the quiescent hepatic stellate cells into myofibroblasts which ultimately promotes fibrosis [19]. Hh ligands are also involved in stimulating ductular reaction (DR) by acting on hepatic progenitor cells (HPC) [20]. These progenitor cells

located in the canals of Hering are bipotentially capable of differentiating into cholangiocytes or hepatocytes [20, 21]. However, in NASH, there is a milieu of chronic inflammation which blocks the hepatocyte differentiation and the cholangiocytes respond to Hh ligands by acquiring a reactive phenotype which is capable of secreting a myriad of chemokines and fibrogenic growth factors that are responsible for advanced liver fibrosis or hepatocellular carcinoma [16].

Liver sinusoidal endothelial cells (LSEC) which play a pivotal role in NASH are also known to both express and respond to Hh signals [22]. LSEC have numerous fenestrations (pores) which help in the transfer of proteins, nutrients and macromolecules between hepatocytes and blood. Capillarization is the process by which endothelial cells lose their fenestra and this plays a key role in development of portal hypertension as well as fibrosis progression. To demonstrate the significance of hedgehog pathway in capillarization, one study, using freshly isolated LSEC from mice, showed that LSEC expressed Hh ligands, Hh receptors and the antagonist (Hhip), and in culture-induced capillarization, there is a significant increase in Hh ligands as well as their target genes [23]. Moreover, inhibition of the Hh signaling pathway was associated with prevention of LSEC capillarization both in vivo and in vitro indicating that these signal channels could be utilized as a potential treatment target against liver fibrosis.

Treatment

Currently, there are no FDA-approved drugs for treatment of fibrosis, however, a number of compounds targeting multiple pathways involved in NASH are currently in phase 2–3 trials. These molecules target one or more pathways that lead to NASH and fibrosis (Fig. 2).

The AASLD recommends the use of vitamin E (800 IU/day) or pioglitazone for treatment against NASH based on results from the PIVENS trial which analyzed 247 patients who had NASH but no diabetes [24]. Both these agents were

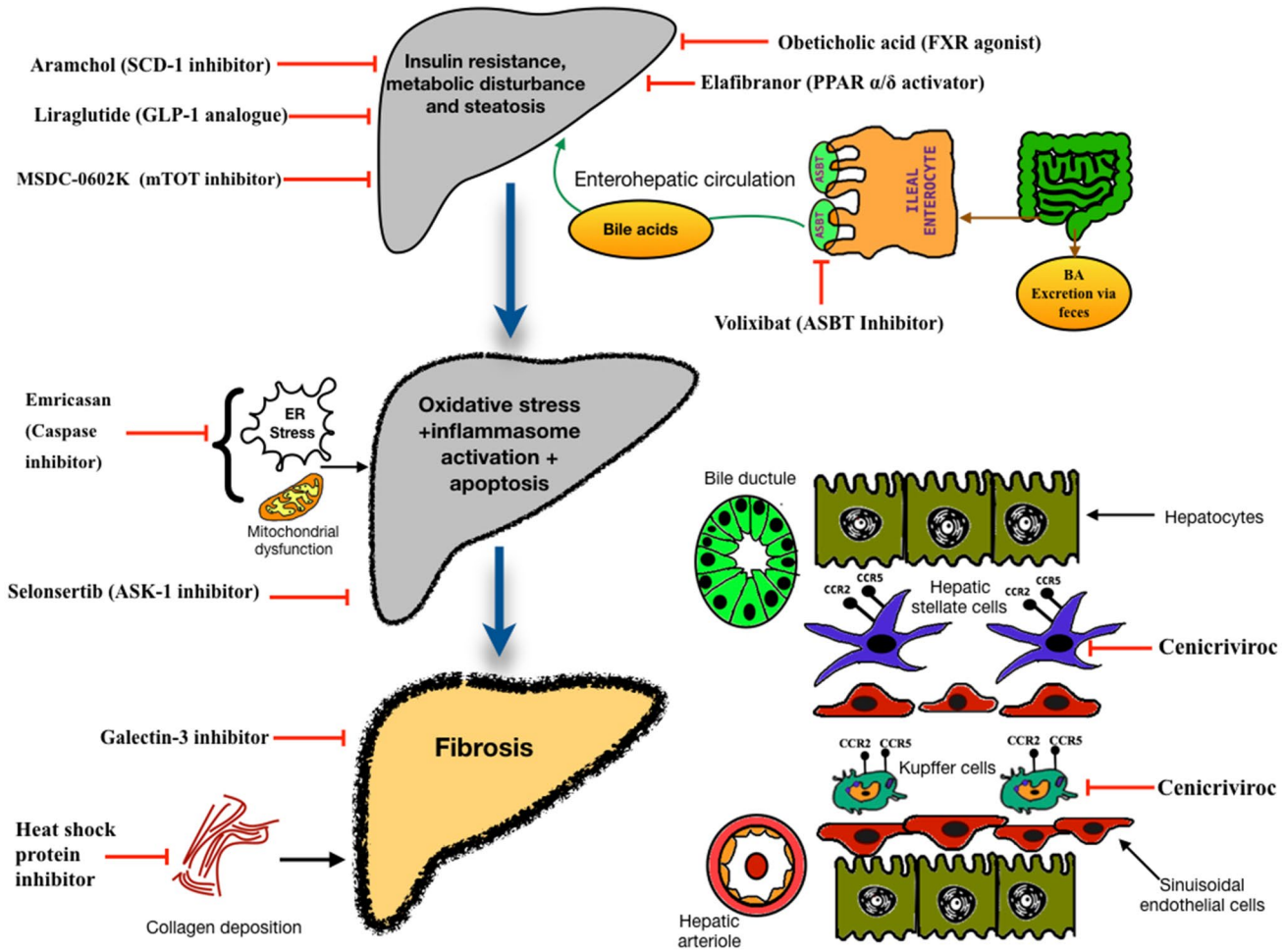

Fig. 2 The pathways that are targeted by drugs currently in clinical trials

associated with reduction in steatosis, lobular inflammation and liver enzymes compared to placebo, however, there was no improvement in fibrosis scores [25]. Since there are reports of increased all-cause mortality and risk of prostate cancer with higher doses of vitamin E, it should be used with caution and perhaps at a lower dose than tested in PIVENS trial [24]. The most common side effect of pioglitazone is weight gain which makes this agent less attractive for the vast majority of NAFLD patients who are overweight.

Four drugs are currently in phase 3 trials and these include cenicriviroc, selonsertib, elafibranor and obeticholic acid. While the trials for cenicriviroc and selonsertib are designed to assess for improvement in fibrosis with no NASH worsening, elafibranor is being evaluated for NASH resolution with no worsening of fibrosis and obeticholic acid is being tested for both improvement in liver fibrosis and resolution of NASH. These drugs and few others in early stages of development will be discussed in this review (Table 1).

Drugs in phase 3 trials

Obeticholic acid (Farnesoid X receptor agonist)

Farnesoid X receptor (FXR), a nuclear hormonal receptor, plays a key role in NASH by regulating carbohydrate and lipid metabolism [26]. In vitro and in vivo studies have shown that FXR is a negative modulator of NF-Kb signaling pathway which is linked to hepatic inflammation and carcinogenesis [27]. Obeticholic acid (OCA) is a FXR agonist that has shown to increase insulin sensitivity, decrease hepatic steatosis and decrease fibrosis in patients with NAFLD [28]. In a phase 2, double blind placebo-controlled trial (FLINT trial) in NASH patients without cirrhosis, 141 patients were randomized to OCA 25 mg daily and 142 patients to placebo for 72 weeks. Of these, 200 patients were available for histological assessment (baseline and 72 weeks liver biopsy) [29]. OCA met the primary outcome (45% vs. 21%, $p=0.002$) of improvement of NAS by ≥ 2 points without worsening of fibrosis. There was also a small improvement (-0.2 vs. 0.1, $p=0.01$) in fibrosis (35% vs. 19%, $p=0.004$) suggesting that this therapy might be beneficial in preventing cirrhosis. The most common side effect was pruritus (23%), and other side effects include elevated total cholesterol or LDL and a decrease in HDL.

Currently, a phase 3 trial (REGENERATE) is enrolling patients with noncirrhotic NASH that are randomized to OCA 10 mg, OCA 25 mg, or placebo with a target completion of the trial in October 2022. The primary end points included superiority in achieving at least one stage of liver fibrosis improvement with no worsening of NASH in patients with stage 2 or stage 3 fibrosis or NASH resolution with no worsening of fibrosis as well as evaluating for all-cause mortality and liver-related outcomes in patients taking OCA compared to placebo (NCT02548351). The interim report of this study was recently published as an abstract (EASL 2019) and as a press release by the manufacturers of OCA [30, 31]. In the 18-month interim primary efficacy analysis, once-daily OCA 25 mg met the primary endpoint of fibrosis (improvement ≥ 1 stage fibrosis with no worsening of NASH ($p=0.0002$ vs. placebo). Although a numerically greater proportion of patients in both OCA treatment arms compared to placebo achieved the primary endpoint of NASH resolution with no worsening of liver fibrosis, the results were not statistically significant. The results of this

Table 1 Drugs currently in various stages of development for the treatment of NASH

Drug name	Mechanism of action	Pharmaceutical company	Phase of clinical trial
Obeticholic Acid (OCA)	Farnesoid X receptor agonist	Intercept pharmaceuticals	3
Selonsertib	Apoptosis signal-regulating kinase-1 inhibitor	Gilead	3
Elafibranor	PPAR α/δ activator	Genfit	3
Cenicriviroc	CCR2/CCR5 antagonist	Allergan	3
Aramchol	Stearoyl-CoA desaturase (SCD-1) inhibitor	Galmed pharmaceuticals	2
GR-MD-02	Galectin-3 inhibitor	Galectin therapeutics	2
MSDC-0602 K	mTOT inhibitor	Cirius therapeutics	2
Emricasan	Caspase inhibitor	Conatus pharmaceuticals	2
Semaglutide	GLP-1 analog	Novo nordisk	2
Volixibat	Apical sodium-dependent bile acid transporter Inhibitor	Mirum pharmaceuticals	2
BMS-986036	Fibroblast growth factor (FGF) 21 analog	Bristol-Myers Squibb	2
MGL-3196	Thyroid hormone receptor (THR) β-selective agonist	Madrigal	2
ND-LO2-s0201	Heat shock protein inhibitor	Bristol-Myers Squibb	1

study are encouraging, but no firm conclusions can be drawn until the final results of this study are published.

Selonsertib (apoptosis signal-regulating kinase-1 inhibitor)

Another drug that had promising phase 2 results is selonsertib, an inhibitor of apoptosis signal-regulating kinase-1 (ASK-1); activation of ASK-1 under oxidative stress may induce hepatocyte apoptosis, inflammation and myofibroblast activation [32]. The phase 2 study enrolled 72 patients with NASH and stage 2–3 fibrosis and randomly assigned them to receive either 6 or 18 mg of selonsertib once daily with or without once-weekly injections of 125 mg of simtuzumab or simtuzumab alone for 24 weeks [33]. At baseline, 65% of the subjects had stage 3 fibrosis and 86% demonstrated severe hepatocellular ballooning. Treatment response was assessed by pre- and post-treatment liver histology and MRI. Patients treated with selonsertib alone had numerically higher rates of fibrosis improvement and lower rates of fibrosis progression compared to patients who received simtuzumab alone; 13 (43%) of 30 patients who received 18 mg and 8 (30%) of 27 who received selonsertib had reduction in fibrosis by at least one stage. However, selonsertib did not have an effect on NASH components.

The promising results of the phase 2 study were not confirmed in phase 3 trials in compensated cirrhosis due to NASH (STELLAR 4) or in those with advanced fibrosis (STELLAR-3). Both these trials that had a randomized large number of patients were discontinued after an interim analysis failed to meet the primary endpoints [34, 35].

Elafibranor (PPAR α/δ activator)

Elafibranor, a dual activator of PPAR α/δ is another potential therapeutic agent that has emerged with favorable results in a recent clinical trial. Peroxisome proliferator-activated receptors (PPARs) are a family of nuclear receptors that are important regulators of energy metabolism, lipid and glucose homeostasis as well as modulators of inflammation [36]. PPAR alpha which is highly expressed in hepatocytes is crucial for fatty acid oxidation, while PPAR δ (also called PPAR b) seen in hepatocytes, stellate cells and Kupffer cells exerts its action by inhibiting the NF-kb pathway leading to downregulation of inflammatory cytokines [36, 37]. In a phase 2 placebo-controlled study (GOLDEN-505) in patients with NASH without cirrhosis, 276 patients were randomly assigned to receive elafibranor either 80 mg or 120 mg daily or placebo [38]. After 52 weeks, there was no significant difference in the primary outcome which was defined as resolution of NASH without worsening fibrosis between elafibranor and placebo. A post hoc analysis, however, based on a modified definition generated positive results. The modified definition defined NASH resolution as disappearance of ballooning along with either disappearance of lobular inflammation or persistence of mild lobular inflammation. In the intention to treat analysis, 120 mg of elafibranor had a significantly higher response rate than placebo (19% vs 12%; $p=0.045$). Moreover, when 15% of the patients with mild NASH were removed from the analysis, 120 mg of elafibranor was significantly superior than placebo indicating that efficacy of elafibranor may be determined by the baseline disease severity. Even though the trial was not designed with antifibrotic goals, it is worth pointing out that among the patients who responded to 120 mg of elafibranor ($n=17$), there was a reduction in fibrosis ($p<0.001$) compared to non-responders of the same regimen. Additionally, elafibranor was associated with improvement glucose and lipid profile, but a mild, reversible increase in creatinine was noted. An ongoing phase 3 trial (RESOLVE-IT) is assessing elafibranor 120 mg vs. placebo in moderate to severe NASH patients with an estimated completion in December 2021 (NCT02704403).

Cenicriviroc (dual antagonist of CCR2 andCCR5)

Chemokines are chemo-attractant cytokines that play a pivotal role in NASH by recruiting neutrophils, monocytes and lymphocytes into areas of inflammation. These chemokines are secreted by hepatic stellate cells, hepatocytes, kupffer cells and endothelial cells and bind onto chemokine receptors present on leucocyte subsets to trigger an inflammatory cascade [39]. Two chemokine ligands that have been studied extensively are CCL2 and CCL5. Studies in animal models have indicated that inhibition of their respective receptors, CCR2 and CCR5, is associated with an improvement in hepatic steatosis and reduction in collagen deposition resulting in decreased fibrogenesis [40, 41]. CENTAUR was a phase 2b randomized double blinded clinical trial designed to evaluate the efficacy and safety of cenicriviroc, a dual antagonist of CCR2/CCR5 [42]. In a phase 2b randomized double blind trial (CENTAUR), 289 patients with NASH and liver fibrosis (stages 1–3) were randomized to cenicriviroc or placebo for 1 year. Compared to placebo ($n=144$), subjects who received cenicriviroc achieved improvement in fibrosis by ≥ 1 stage and no worsening of steatohepatitis (20% vs. 10%; $p=0.02$). Subjects with higher baseline disease activity (NAS ≥ 5, prominent hepatocyte ballooning and moderate to severe fibrosis) had the highest treatment benefit. Despite these encouraging signs, the primary end point (improvement in NAS) was not met in this phase 2 trial. However, given the pressing need for antifibrotic medications in NASH, a phase 3 trial (AURORA) has started recruiting patients with F2–F3 fibrosis with estimated primary completion in September 2020 (NCT03028740).

Drugs in early stages of development

Emricasan (caspase inhibitors)

Caspases are proteolytic enzymes that are activated by two apoptotic pathways: extrinsic (initiated by death receptors) and intrinsic (ER stress and mitochondrial dysfunction) that trigger hepatocyte apoptosis which sets the stage for NASH progression and fibrogenesis [43]. Emricasan, an irreversible pan-caspase inhibitor demonstrated beneficial therapeutic effects in murine models with diet-induced NASH by reducing liver injury, hepatocyte apoptosis and attenuation of hepatic stellate cell activation and fibrogenesis [44]. The efficacy of 25 mg of twice daily emricasan was evaluated in a phase 2 trial in 86 patients with Child–Pugh A or B cirrhosis of varying etiology (23% attributed to NASH) [45]. Even though there was a reduction in the level of cleaved cytokeratin-18 (a caspase-related biomarker) at 3 months compared to baseline, statistical significance (of what? What was the primary end point?) was not achieved ($p = 0.092$). However, a number of secondary endpoints were met such as improvement in MELD scores in subjects with NASH cirrhosis ($p = 0.029$) compared to placebo as well as reduction in total bilirubin, albumin, INR and Child–Pugh scores among subjects who had a baseline MELD > 15. Another multi-center study analyzed the effects of emricasan in a small group of patients ($n = 23$) with compensated cirrhosis secondary to NASH and hepatitis C, and demonstrated a clinically significant reduction ($p = 0.003$) in HVPG in 12 subjects with severe portal hypertension (HVPG ≥ 12 mmHg) after 28 days of treatment [46]. 4 out of the 12 patients had ≥ 20% decrease compared to baseline and 8/12 subjects had ≥ 10% decrease from baseline. However, in the overall group of patients with portal hypertension corresponding to HVPG ≥ 5 mm Hg, no significant reduction was seen. There was also a reduction in the markers of apoptosis such as caspase 3/7 and cleaved cytokeratin 18 compared to baseline after treatment with emricasan. ENCORE-NF(NCT02686762) and ENCORE-PH (NCT02960204) are two phase 2b trials that are currently ongoing to evaluate the efficacy of emricasan in patients with NASH fibrosis and NASH cirrhosis, respectively, and these trials expected to be completed in the near future.

Liraglutide (GLP-1 analog)

Liraglutide, a long-acting GLP-1 analog that increases insulin secretion from pancreatic beta cells has been approved by the FDA for the treatment of type 2 diabetes since 2010. Recently, this agent has gained interest as a potential therapeutic option against NASH based on results from a placebo-controlled phase 2 study that involved 52 patients with NASH who were randomly assigned to receive liraglutide for 42 weeks (LEAN trial) [47]. The trial showed resolution of NASH with no worsening of fibrosis in 9 (39.1) of 23 patients in the treatment group and 2 (9%) of 22 in the placebo ($p = 0.019$). Moreover, only 2 of 23 patients in the treatment group had progression to fibrosis compared to 8 of 22 in the placebo group ($p = 0.04$). Semaglutide, a similar GLP-1 analog is currently being investigated in a phase 2 trial involving NASH patients, the primary outcome being NASH resolution without worsening fibrosis and the study is anticipated to be completed in 2020 (NCT02970942).

Aramchol (stearoyl-CoA desaturase (SCD-1) inhibitor)

Aramchol is a stearoyl-CoA desaturase (SCD-1) inhibitor which had promising results in a phase 2 clinical trial that was completed recently. SCD plays a key role in regulating fatty acid metabolism and is responsible for converting saturated fatty acids into monounsaturated fatty acids [48]. Studies in experimental murine models have demonstrated that inhibition of SCD-1 leads to improvement in triglyceride accumulation, and attenuation of liver injury, hepatocellular degeneration and inflammation [49]. In a phase 2 trial (ARREST) that enrolled 247 patients, 60% of whom had stage 2 or stage 3 fibrosis at baseline, NASH resolution without worsening fibrosis was seen in 19.2% of subjects who were treated with 600 mg of aramchol ($n = 78$) compared to 7.5% in placebo ($n = 40$, OR = 4.74, $p = 0.051$) [50]. Additionally, the drug was also associated with an improvement in fibrosis stage (≥ 1 stage) without worsening of NASH compared to placebo (29.5% vs 17.5%, OR = 1.88, $p = 0.21$). A much larger study may be necessary to determine whether aramchol is likely to be beneficial in NASH.

Galectin-3 inhibitor

Galectin-3 (gal-3), a beta-galactoside-binding lectin expressed in kupffer cells, septal/portal macrophages and hepatocytes, has a critical role in the pathogenesis of liver fibrosis [51]. In a preclinical study, when rats with toxin-induced fibrosis and cirrhosis were treated with inhibitors of galectin, there was a reduction in collagen deposition, bridging fibrosis and reversal of cirrhosis [52]. Moreover, treatment was associated with a significant reduction in portal hypertension. A phase 2b clinical trial to evaluate the efficacy of GR-MD-02 (inhibitor of gal-3) in patients with NASH cirrhosis and hepatic venous pressure gradient

(HVPG) > 6 mm of Hg failed to achieve a statistically significant reduction in HVPG in the treated group compared to the placebo [53]. However, a post hoc analysis reported statistical significance in a subset of patients who did not have esophageal varices at baseline (which was 50% of the study population) when treated with GR-MD-02 at a dose of 2 mg/kg compared to placebo ($p < 0.01$), but the improvement was not seen with the higher doses (8 mg/kg). Treatment has no effect on liver fibrosis or NAS score, but a statistically significant improvement in hepatocyte ballooning was observed. Moreover, fewer patients on active treatment developed varices at the end of the study compared to placebo (6/33 in the placebo group vs. 0/25 among patients on 2 mg/kg of GR-MD-02 and 1/23 in patients on 8 mg/kg, $p = 0.01$). Based on these data, a phase 3 trial has been planned [54].

Heat shock protein inhibitor

Heat shock protein (HSP47) is a collagen-binding molecular chaperone located in the endoplasmic reticulum that is essential for collagen fibril formation and collagen deposition which are key elements of fibrogenesis [55]. The progression of liver fibrosis is significantly reduced in mouse models that lack HSP47 [55]. When vitamin A-coupled liposomes carrying small interfering RNA (siRNA) against gp46 (rat homolog of HSP47) were intravenously injected into animal models with cirrhosis, rapid resolution of liver fibrosis was seen [56]. ND-LO2-s0201, a lipid nanoparticle that targets hepatic stellate cells and capable of reversibly inhibiting HSP47 was tested in a phase 1 trial on healthy subjects ($n = 20$) and was generally well tolerated and this may be tested further in phase 2 trials [57].

MSDC-0602K (thiazolidinediones)

MSDC-0602K, a new-generation thiazolidinedione that binds on mitochondrial pyruvate carrier (MPC) is another drug in the pipeline that targets metabolic pathways early in the disease course and could potentially emerge as a treatment option for NASH in the future [58]. Unlike traditional insulin sensitizers (pioglitazone and rosiglitazone), this drug does not fully activate PPARγ, hence it has a limited effect on weight gain [58]. The beneficial pharmacological effects of MSDC-0602K were seen in mice models that were fed trans-fatty acids, fructose, and cholesterol to induce liver injury and fibrosis [59]. The treatment with MSDC-0602K was shown to prevent and reverse liver fibrosis, reduce toxic lipid accumulation and induce reversal of hepatic stellate cell activation. A phase 2 clinical trial is currently underway to evaluate efficacy and safety of MSDC-0602K in patients with biopsy-proven NASH and fibrosis (and no cirrhosis) and is expected to be completed by June 2019. The primary outcome will be histological improvement in NAS (decrease is at least 2 points) with no worsening of fibrosis and secondary outcome would include improvement in fibrosis by at least 1 stage (CRN staging) without worsening of NASH (NCT02784444).

Volixibat (apical sodium-dependent bile acid transporter inhibitor)

Apical sodium-dependent bile acid transporter (ASBT) is a transmembrane protein located in the terminal ileum responsible for reabsorption of bile acids (BAs) from the gastrointestinal tract and transporting them back into the liver via enterohepatic circulation [60]. Inhibition of this transporter will result in increased excretion of BAs through feces and reduced recirculation of BAs back into the liver, ultimately facilitating removal of free cholesterol from the liver. Since accumulation of free fatty acids is critical in the pathogenesis of NASH, inhibition of ASBT could have a potential role as a therapeutic agent. Preclinical studies have demonstrated that when murine models of NASH are treated ASBT inhibitors, there is an improvement in the NAS score as well as reduction in hepatic triglyceride and cholesterol concentrations [61]. The efficacy of volixibat, an ASBT inhibitor, to reduce NAS score by at least 2 points was explored in a phase 2 trial by comparing it with placebo for 48 weeks. Unfortunately, interim analysis after 24 weeks reported a reduction neither in the NAS score nor in the ALT levels. There was also no improvement in hepatic steatosis which was measured using MRI proton density fat fraction [62].

BMS-986036 (fibroblast growth factor [FGF] 21 analog)

FGF21 is an important regulator energy metabolism, and it is produced in the liver where it reduces glucose production and lipogenesis, and increases fatty acid oxidation. Upregulation of FGF21 seen in NAFLD is thought to be an insufficient compensatory mechanism. A recent phase 2 study suggested that administration of parenteral FGF may be beneficial in NASH patients (NCT02413372) [63]. In this small study, 49 patients were randomized to receive FGF21 and 26 patients placebo for 16 weeks. Patients who received FGF21 showed significant improvement in steatosis compared to placebo, and the drug was well tolerated. A phase 2b trial is currently in progress.

MGL-3196 (thyroid hormone receptor (THR) β-selective agonist)

In a phase 2 study, MGL-3196 (thyroid hormone receptor (THR) β-selective agonist developed by Madrigal) showed improvement in steatosis, hyperlipidemia and possible improvement in fibrosis [64]. If phase 3 trials confirm its efficacy, it may prove to be a useful drug in those with hyperlipidemia and NAFLD.

In addition to the above drugs, there are many other molecules in early trial phases. Many of these drugs may progress to phase 2 or 3 trials in the future.

Combination treatments

Utilizing a combination of drugs to target the multiple pathways responsible for NASH pathogenesis is another option that could be explored in the future. Experimental studies on murine models combining selonsertib and simtuzumab had encouraging results, however, a phase 2 trial that analyzed this combination on patients with stage 2 and stage 3 fibrosis (described earlier in this review) did not find the combination to be very effective [33, 65]. Another small study combined acetyl-coenzyme carboxylase (ACC) inhibitor-GS-0976, capable of inhibiting de novo lipogenesis along with FXR agonist GS-9674 on 20 patients with NASH and fibrosis (stage 2 and stage 3) and demonstrated that combination therapy resulted in improvement of hepatic steatosis and liver stiffness, as measured by magnetic resonance proton density fat fraction (MRI-PDFF) and magnetic resonance elastography (MRE), and a reduction in other markers of fibrosis [66]. A proof of concept study tested the combination of elafibrinor (PPAR α/δ agonist) and nitazoxanide on animal models of NASH and fibrosis, and showed that the combination therapy produced a synergistic action on multiple pathways, resulting in inhibition of hepatic stellate cell activation as well as significant reduction in liver fibrosis [67]. Another experimental study used elafibrinor as an universal backbone and combined other agents such as obeticholic acid, GS-0976, selonsertib or cenicriviroc to assess their synergistic activity [68]. They found a significant improvement in NAS score by 3 points with a combination of elafibrinor and GS-0976, and also a combination of elafibrinor and cenicriviroc, but not with other drug combinations. A significant reduction in fibrosis was seen with the addition of obeticholic acid and selonsertib to elafibrinor. Developing a combination therapeutic strategy to tackle NASH should ideally involve a drug that targets the metabolic component (de novo lipogenesis and insulin resistance) along with other agents that can attenuate inflammation and/or fibrosis [69].

Challenges in assessing fibrosis in clinical trials

NASH is a heterogeneous disease with varying severity and progression rates seen in a population with multiple comorbidities and risk factors. The major challenge in the treatment trials is identifying reliable markers of improvement in fibrosis within a defined time period in a disease with a long natural history. Moreover, the previous clinical trials have shown improvement in histology in up to 20% of patients who were randomized to placebo. Although liver biopsy remains the gold standard, it is also associated with potential complications and sampling errors. Additionally, patients are reluctant to undergo repeated biopsies in placebo-controlled trials. Despite the above limitations, there is a general agreement that there should be at least 1 stage or more improvement in fibrosis by histology without worsening of steatohepatitis (by NAS score). It has been suggested that MR elastography (MRE) could replace liver histology, but there is a paucity of data to support the use of MRE instead of liver histology for primary endpoint assessment of fibrosis for registration trials. It is perhaps preferable to use MRE as a supporting endpoint until we have more data. In those with advanced fibrosis, the ideal endpoint is reduction in complications of liver disease such as portal hypertensive bleeding, ascites, encephalopathy or liver cancer. To use complications as primary endpoints will require a large sample size and a longer study duration. This is not a pragmatic strategy for a variety of reasons including costs and patient fatigue. Until we have more data, therefore, liver histology will remain the gold standard despite some of its limitations.

Conclusions

Currently, there are no approved medications for patients with NAFLD and fibrosis. The mainstay of treatment is moderate intensity exercise along with a hypocaloric diet to achieve weight loss, as this has shown to improve hepatic steatosis. Bariatric surgery is an option in obese patients who do not respond to traditional lifestyle modifications, however, data are lacking regarding its long-term efficacy. There exists a critical need to develop effective pharmacotherapy against fibrosis given the magnitude of its clinical implications. Since the pathogenesis of NASH is complex and involves multiple pathways, a combination of pharmacological agents may be required to tackle the problem rather than a single agent. Early results from some phase 2 and phase 3 trials are encouraging and we believe that therapeutic agents which can halt or improve fibrosis may be available in the near future.

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Compliance with ethical standards

Conflict of interest Joseph J. Alukal and Paul J. Thuluvath declare that they have no competing interests.

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