Advances and challenges in the management of advanced fibrosis in nonalcoholic steatohepatitis

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Abstract: Nonalcoholic fatty liver disease (NAFLD) is rapidly becoming the most common type of chronic liver disease worldwide. From the spectrum of NAFLD, it is nonalcoholic steatohepatitis (NASH) that predominantly predisposes patients to higher risk for development of cirrhosis and hepatocellular carcinoma. There is growing evidence that the risk of progression to cirrhosis and hepatocellular carcinoma is not uniform among all patients with NASH. In fact, NASH patients with increasing numbers of metabolic diseases such as diabetes, hypertension, visceral obesity and dyslipidemia are at a higher risk of mortality. Additionally, patients with higher stage of liver fibrosis are also at increased risk of mortality. In this context, NASH patients with fibrosis are in the most urgent need of treatment. Also, the first line of treatment for NASH is lifestyle modification with diet and exercise. Nevertheless, the efficacy of lifestyle modification is quite limited. Additionally, vitamin E and pioglitazone may be considered for subset of patients with NASH. There are various medications targeting one or more steps in the pathogenesis of NASH being developed. These drug regimens either alone or in combination, may provide potential treatment option for patients with NASH.

Keywords: Nonalcoholic fatty liver disease; nonalcoholic steatohepatitis; pathogenesis; noninvasive; vitamin E; pioglitazone

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. It encompasses a spectrum, ranging from simple steatosis, which is frequently nonprogressive, to nonalcoholic steatohepatitis (NASH), which is the progressive form of NAFLD leading to advanced fibrosis and cirrhosis. The prevalence of NAFLD has increased substantially, likely due to the obesity epidemic, and shown to be around 25% in the general population worldwide. However, patient populations such as those with morbid obesity undergoing bariatric surgery can have a prevalence rate for NAFLD as high as 90%. In contrast, not all patients with NAFLD are overweight or obese and, in fact, about 8–10% of NAFLD patients have a body mass index (BMI) level of less than 25 and are considered lean.9

Current literature reports that 10–20% of patients with NAFLD have NASH. Individuals with NASH are primarily at an increased risk of developing advanced fibrosis, cirrhosis and hepatocellular carcinoma (HCC). NASH has already become the second leading indication for liver transplantation, and expected to be the top indication for liver transplant listing in a decade or two.10 Because of the risk of progressive course of NASH, patients with NASH should be considered for treatment. This is especially urgent for NASH patients with significant fibrosis. In fact, NASH patients, especially those with fibrosis, are the focus of most active clinical trials.11,12

Before discussing the experimental treatment options for NASH, it is important to understand its underlying pathogenesis and pathways that lead to hepatic steatosis, hepatocyte injury and fibrosis in NASH.

Mechanisms and effects of hepatic steatosis

The key feature of NAFLD is the presence of macrovesicular steatosis, however the addition of...
hepatic inflammation, ballooning (with or without Mallory’s hyaline), and varying degrees of fibrosis are needed to define NASH. Given the close association between NAFLD and metabolic syndrome components, insulin resistance has been the focus of the pathogenic mechanism of NASH. Historically, the ‘two-hit’ hypothesis was proposed to explain the mechanisms underlying the pathogenesis of NASH. As our understanding of the phenotype NASH increased, it is highly likely that the pathogenesis involves multiple pathways with potentially multiple hits. Nevertheless, it must be noted that the mechanisms underlying development of NASH are complex and still not fully understood.

In the context of NAFLD, it is proposed that hepatic steatosis is caused by the accumulation of lipid in hepatocytes secondary to multiple factors, including environmental, metabolic, and genetic causes, which may be occurring simultaneously. In most cases, insulin resistance results in an increase in lipolysis in the adipose tissue, leading to increased free fatty acid mobilization to the bloodstream and increased influx of free fatty acids to hepatocytes. After the uptake of free fatty acids in the liver, they are used to form very-low-density lipoprotein particles, which are released back to bloodstream, and form triglycerides in hepatocytes, thus causing increased triglyceride storage in the liver. This increased lipid content of hepatocytes may not only cause an increase in the oxidative stress, but also results in increased β-oxidation of free fatty acids in mitochondria, and continuing the cascade as increased oxygen species, further causing lipid peroxidation and activating cellular oxidative stress. Kupffer cells and stellate cells play an important role in progression from NAFLD to NASH, as the activation of stellate cells promote fibrogenesis. In fact, the recruitment of extra-hepatic inflammatory cells to the site of inflammation is mainly mediated by the interactions between the chemokines and cytokines that were secreted by activated stellate cells and Kupffer cells, and their ligands. Secondary to hepatic steatosis, activated stellate cells and Kupffer cells secrete cytokines like tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), IL-1β, as well as chemokines like CCR2 and CCR5 and their ligands, CCL2 and CCL5, respectively. These molecules promote monocyte and macrophage recruitment and their infiltration to tissues. Indeed, these monocytes and macrophages, with activated stellate cells, are the main source of transforming growth factor beta (TGFβ), which triggers collagen production by activating stellate cells and promoting fibrogenesis.

In terms of organelle-based oxidative stress, three distinct signal transduction pathways are of particular importance in the endoplasmic reticulum: inositol requiring (IRE) 1α, protein kinase RNA (PKR)-like endoplasmic reticulum (ER) kinase (PERK) and activating transcription factor (ATF) 6α. When these molecules sense increased levels of endoplasmic-reticulum stress, they trigger a compensatory mechanism, called unfolded protein response. This overwhelmed unfolded protein response in the setting of excess oxidative stress can initiate cell-death cascade. Additionally, chronic endoplasmic reticulum stress produces more reactive oxygen species that triggers hepatocyte inflammation through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and jun-(N)-terminal kinase (JNK) pathways. Another key organelle is the mitochondria, whose functions are disrupted by increased lipid metabolism and increased hepatic steatosis. Another important factor is related to the intestine–liver axis. Studies have demonstrated that ingestion of high-fat diet results in impairment of intestinal barrier function. This can lead to the leakage of bacterial products, most importantly lipopolysaccharides, to the bloodstream. These molecules have been found to play a role in the development of hepatic steatosis and inflammation through toll-like receptors.

Excessive lipid accumulation in liver causes both cellular-based and organelle-based oxidative stress. Kupffer cells and stellate cells play an important role in progression from NAFLD to NASH, as the activation of stellate cells promote fibrogenesis. In fact, the recruitment of extra-hepatic inflammatory cells to the site of inflammation is mainly mediated by the interactions between the chemokines and cytokines that were secreted by activated stellate cells and Kupffer cells, and their ligands. Secondary to hepatic steatosis, activated stellate cells and Kupffer cells secrete cytokines like tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), IL-1β, as well as chemokines like CCR2 and CCR5 and their ligands, CCL2 and CCL5, respectively. These molecules promote monocyte and macrophage recruitment and their infiltration to tissues. Indeed, these monocytes and macrophages, with activated stellate cells, are the main source of transforming growth factor beta (TGFβ), which triggers collagen production by activating stellate cells and promoting fibrogenesis.
β-oxidation. Both endoplasmic reticulum stress and mitochondrial dysfunction lead to apoptosis and fibrosis, which are key elements of NASH. These processes leading to development of hepatic steatosis and NASH have increasingly gained popularity and have become the target for the future therapeutic options in NASH.

Detection of fibrosis among patients with nonalcoholic fatty liver disease and nonalcoholic steatohepatitis

As stated above, NASH is the progressive form of NAFLD, and individuals with advanced fibrosis are at a greater risk for adverse outcomes in the long term. Although the gold standard for diagnosing NASH and stage of fibrosis is through histological evaluation by a liver biopsy, research has been focused on developing non-invasive assessment of fibrosis, either via imaging techniques or serum biomarkers and the panels utilizing those biomarkers.

Imaging

Traditional imaging modalities like ultrasound, computerized tomography and magnetic resonance imaging (MRI) have been used for the diagnosis of NAFLD, however, those modalities are unable to diagnose NASH or stage hepatic fibrosis. On the other hand, a number of new technologies have assessed tissue stiffness as a surrogate of hepatic fibrosis. In this context, transient elastography (TE), a non-invasive method that measures liver stiffness, has been shown to estimate hepatic fibrosis. Another method is acoustic radiation force impulse (ARFI), which integrates elastography and conventional B-mode ultrasonography. Different from TE, ARFI targets a slightly larger region of interest in liver parenchyma. Different studies have shown an area under the receiver operating characteristics curve (AUROC) between 0.86 and 0.94 for mild to moderate fibrosis (F1 and F2), and 0.90–0.98 for advanced fibrosis (F3 and above). While these two modalities focus on a relatively small part of liver, another technology, magnetic resonance elastography (MRE) is able to analyze the entire liver. MRE requires the addition of a special software to MRI, and studies demonstrated that MRE results are highly accurate for detecting liver fibrosis, with AUROC values ranging between 0.96 and 0.99. Although MRE is more accurate, it may not be easily accessible by most experts. In contrast, TE may be slightly less accurate in estimating stage of liver disease but is portable and easily accessible as a point-of-care modality.

Serum markers and biomarker panels

There are multiple serum markers and biomarker panels for the use of healthcare providers when assessing liver fibrosis in a non-invasive fashion. Among them, the most extensively validated scoring system has been the NAFLD fibrosis score (NFS), which includes hyperglycemia, BMI, platelet count, albumin, aspartate transaminase (AST)/alanine transaminase (ALT) ratio and age. NFS is accurate in distinguishing the severity of liver fibrosis and reportedly can avoid liver biopsy in three of four patients with NAFLD. The challenge with NFS is that almost half of the patients fell in between the two cutoff points. Another widely used scoring system is FIB-4, which was originally developed to stage liver disease among patients with chronic hepatitis C virus (HCV) infection. FIB-4 uses easy-to-obtain serum markers (age, platelet count, AST and ALT levels); calculations are simple, and the results are available immediately. FIB-4 has an AUROC of 0.85–0.87 for advanced fibrosis and a value < 1.45 can exclude fibrosis with 90–98% certainty. It is possible that the FIB-4 index is more useful in mostly advanced fibrosis cases where it can reduce the number of liver biopsies. Similar to the FIB-4 index, AST/platelet ratio (APRI) was first used in patients with chronic HCV infection to stage their liver disease severity. Previous studies demonstrated the sensitivity and specificity of APRI score > 1 for significant fibrosis were 30% and 92.8%, respectively. And, like other panels, APRI may be used to exclude significant fibrosis. Beside these most commonly used biomarker panels, multiple other panels have been developed. Among those, Fibrotest (BioPredictive S.A.S, 218 Boulevard Saint-Germain, 75007 Paris, France) combines five biochemical markers (haptoglobin, α2-macroglobulin, apolipoprotein a1, total bilirubin and gamma glutamyl transferase) and adjusts according to age and sex. FibroTest has an AUROC of 0.84 for detecting advanced fibrosis, however for distinguishing minimal fibrosis from intermediate fibrosis, FibroTest falls short. With the advances in proteomics technology, new biomarkers have been searched for non-invasive
assessment of NASH, and specifically, glycoproteomics has been receiving considerable attention in this context. The examples of these markers include fucosylated haptoglobin, Mac-2 binding protein and fetuin-A. Fucosylated haptoglobin was proposed as a useful marker for detecting NASH and advanced fibrosis and studies demonstrated AUROC of 0.73 and 0.72, respectively.44 In a similar fashion, Mac-2 binding protein had an AUROC of 0.81 for detecting NASH, and its level showed close correlation with the severity of fibrosis and hepatocyte ballooning.45 Another study from Japan demonstrated that the serum Fetuin-A level, which is a liver glycoprotein, was significantly and negatively correlated with liver fibrosis.46 Although these markers seem promising for noninvasive assessment of NASH and fibrosis, they are still at the investigational level and not clinically available. In addition to biomarkers of fibrosis, there has been some attempt to find biomarkers for NASH. In this context, cytokeratin-18 (CK-18) is another protein which was suggested to have increased levels in transition from simple steatosis to NASH and fibrosis. Although these markers seem promising for noninvasive assessment of NASH and fibrosis, they are still at the investigational level and not clinically available. In addition to biomarkers of fibrosis, there has been some attempt to find biomarkers for NASH. In this context, cytokeratin-18 (CK-18) is another protein which was suggested to have increased levels in transition from simple steatosis to NASH and fibrosis. Although some studies reported that CK-18 could be an independent predictor of NASH, with an AUROC of 0.83,47 there is no exact cutoff point for NASH or advanced fibrosis, and the test is currently not available in daily clinical practice.

Some of these biomarkers are involved in the fibrogenesis and fibrolysis processes. Hyaluronic acid (HA), which is a component of extracellular matrix, was among the first serum biomarkers shown to correlate with hepatic fibrosis. However, it was shown that the levels of HA change according to fasting status and show large within-individual variations, suggesting that it might not be a highly reliable marker.48 Another panel is called enhanced liver fibrosis (ELF), which combines age with three markers of matrix turnover, including HA. The ELF panel was modified later with the addition of five markers (including BMI) and its diagnostic accuracy improved further, with AUROCs of 0.98, 0.93 and 0.84 for severe, moderate and no fibrosis, respectively.49 Hepascore (HS; Quest Diagnostics, Madison, NJ, USA) uses six variables and seems to be slightly more complex than other scoring systems. It has an AUROC of 0.90 for detecting cirrhosis and high positive predictive value for advanced fibrosis.50

Although non-invasive tests have been developed to offer alternatives for staging fibrosis, the diagnosis of NASH is still based on histological examination of liver biopsy. However, liver biopsy has its own challenges and limitations, including invasiveness of the procedure, potential sampling errors, and inter- and intraobserver variability.51

**Identifying high-risk populations for nonalcoholic fatty liver disease, nonalcoholic steatohepatitis and advanced fibrosis**

As stated above, the prevalence of NAFLD has been rising and the disease is affecting a quarter of the general population.5 Parallel to this, the number of patients with NASH is also increasing. It is estimated that the prevalence of NASH in the general population ranges between 1.5% and 6.5%.5 However, some individuals with certain metabolic conditions are at a higher risk for the development of NAFLD and progression to NASH. In this context, conditions that have well-established associations with NAFLD include visceral obesity, type II diabetes, dyslipidemia, polycystic ovary syndrome, obstructive sleep apnea, and hypothyroidism.52,53 As these conditions are highly prevalent in the general population, it is a challenge to identify NAFLD patients at high risk for progression to advanced disease stages. Among patients with NAFLD, the presence of components of metabolic syndrome is a strong predictor of NASH. In fact, studies demonstrated that as the number of metabolic syndrome components increases, the risk of progression to advanced fibrosis and mortality will increase in parallel.54,55 At this point, the aforementioned non-invasive tools, combination of serum markers and imaging modalities are extremely helpful in identifying patients with higher likelihood of having stage 3 and stage 4 fibrosis, which is cirrhosis. In fact, the current American Association for the Study of Liver Disease guidelines recommend the use of NFS, FIB-4 index, TE and MRE in clinical practice (if available) to identify patients with high risk for NASH and fibrosis.7

Another important point when identifying patients who have high risk for NASH and advanced fibrosis is screening for complications of cirrhosis such as HCC and esophageal varices. It is well known that the presence of NAFLD is a risk factor for the development of HCC. Progression to cirrhosis due to NASH will increase the risk of HCC with an incidence rate
between 1% and 8%.\(^5\) Kawamura and colleagues reported that patients with NAFLD and advanced fibrosis had a 25-fold increased risk for the development of HCC compared with patients with NAFLD without fibrosis.\(^5\) Current guidelines recommend that patients with NASH-related cirrhosis should undergo HCC screening with ultrasound, with or without α-fetoprotein, every 6 months.\(^5\) Because the risk of HCC is very low in patients with NAFLD without cirrhosis, surveillance is not recommended for that cohort.\(^5\) In addition to HCC screening, other recommendations for patients with cirrhosis (screening for varices, avoidance of hepatotoxic drugs, etc.) should apply to these patients.

The current approach to nonalcoholic steatohepatitis treatment

Nonpharmacological options

Currently, lifestyle modification is the first-line recommendation for treatment of NASH. In this context, lifestyle modification should lead to sustained weight loss. Although diet and exercise can lead to weight loss, less than 15% of patients can maintain the weight loss in the long run.\(^1\)\(^,\)\(^5\) Furthermore, a small amount of weight loss (3–5%) can lead to improvement of hepatic steatosis. On the other hand, 7–10% weight loss is required to see improvement of necroinflammation and fibrosis in NASH.\(^5\)\(^,\)\(^6\) Additionally, exercise may also play a beneficial role. It was recently shown that either aerobic or resistance exercise, in moderate intensity, 3–4 times a week, 20–40 min per session is ideal for fat mobilization from the liver.\(^6\) It should be noted that increased physical activity should be accompanied by a healthier diet in order to achieve the desired goals. As noted previously, the sustainability of weight loss through lifestyle modification is quite limited.

Pharmacological options

Because of the limitations of lifestyle modifications, it has been imperative to develop drugs as potential treatment regimens for NASH. In this context, agents targeting various steps in the pathogenesis of NAFLD and NASH have been studied, and in fact, many of them are still being investigated in phase III clinical trials as listed below.

One targeted mechanism in NASH treatment has been the oxidative stress and inflammation. One of the well-known agents has been vitamin E, which is a free-radical scavenger and protects the structural components of the cell membrane from peroxidation.\(^6\)\(^,\)\(^6\) In fact, in biopsy-proven NASH patients, who are free of diabetes and cirrhosis, vitamin E is a recommended treatment.\(^6\)

Tumor necrosis factor alpha (TNF-α), also known as cachexin, is another molecule responsible for inflammation, apoptosis and fibrosis of hepatocytes.\(^6\) A methylxanthine derivative, pentoxifylline (PTX), works as a phosphodiesterase inhibitor and decreases TNF-α gene transcription. Previous studies demonstrated the effect of PTX on histologic features of NASH. When compared with placebo, PTX significantly improved lobular inflammation and fibrosis in patients with biopsy-proven NASH.\(^6\) However, in the same study, patients with NASH-related cirrhosis were excluded, the number of patients with diabetes was very small and there were no differences in secondary outcomes.\(^6\) Moreover, a meta-analysis by Du and colleagues showed that PTX can lower transaminase levels and improve lobular inflammation.\(^6\) Even though PTX seems promising with the available data, larger clinical trials are needed to support the use of PTX in patients with NASH.

Beside the TNF-α pathway, production of inflammatory cytokines, chemokines and promotion of apoptosis can also be triggered by activation of apoptosis signal-regulating kinase-1. It was suggested that inhibition of this pathway could be effective for the management of advanced fibrosis among patients with NASH. In this context, selonsertib, which is an apoptosis signal-regulating kinase-1 inhibitor, was studied among patients with NASH in a phase II study and found to be safe and potentially effective.\(^6\) STELLAR 3 and 4 are larger phase III trials to assess the efficacy of selonsertib in patients with NASH who have advanced fibrosis.

As stated above, the activation of stellate cells has been well known as a key factor for fibrogenesis. The mechanisms mediating the inflammatory immune response that lead to fibrogenesis have been targets for the management of advanced fibrosis in NASH. In this context, inhibition of C-C chemokine receptor types 2 and 5 (CCR2 and CCR5) have been thought to result in a decrease in recruitment and migration of pro-inflammatory monocytes to the liver, thus decreasing the inflammatory response and degree...
of fibrosis. An immunomodulatory agent, cenicriviroc, can inhibit both CCR2 and CCR5, and animal studies already demonstrated its potent anti-inflammatory and antifibrotic activities. In fact, early-stage clinical trials of cenicriviroc showed promising antifibrotic effect among non-cirrhotic NASH patients. This drug is also being tested in a large phase III clinical trial (AURORA).

Another important mechanism for the development of NAFLD and NASH is insulin resistance. For this reason, different types of insulin sensitizers have been studied for the treatment of these conditions. In fact, pioglitazone and rosiglitazone, which are members of thiazolidinedione family and work through peroxisome-proliferator-activated receptor gamma (PPAR-γ), were studied among patients with NASH. Previous studies demonstrated that patients with NASH, who were treated with thiazolidinediones may show improvement in hepatic steatosis, inflammation and hepatocyte ballooning, albeit the data on hepatic fibrosis were not clear. A recent clinical trial of pioglitazone in diabetic or prediabetic patients with NASH showed improvement of all histologic components of NASH. Finally, a recent meta-analysis confirmed these benefits. These data provided the underlying evidence for the consideration of pioglitazone for some patients with NASH.

In addition to PPAR-γ, other members of the PPAR family play different roles in the pathophysiology of NAFLD and NASH, thus being targeted in the management of these conditions. PPAR-α activation inhibits inflammatory genes induced by NF-κB and decreases the expression of acute-phase response genes. Similarly, PPAR-δ exerts anti-inflammatory activities in macrophages and Kupffer cells. For this reason, activating this pathway by using a PPAR-α/δ agonist was suggested as an option for the management of NASH. In this context, elafibranor, which is a PPAR-α/δ agonist, was compared with placebo in a phase II study among patients with NASH but without cirrhosis. Post hoc analysis of the elafibranor 120 mg daily arm showed promising results and a larger, phase III clinical trial (RESOLVE-IT) is currently underway to evaluate the long-term outcomes of elafibranor on stage 1–3 fibrosis among patients with NASH.

In addition to those agents in the PPAR family, a number of other agents are being tested. In this context, saroglitazar, a PPAR-α/γ agonist which has been shown as effective in the treatment of diabetic dyslipidemia, is currently being studied in a phase II clinical of NASH. Another agent that works through PPAR receptors is MSDC-0602, which has similar insulin-sensitizing effects as rosiglitazone but does not increase osteoclast number, thus not causing osteoporosis. MSDC-0602 is being studied in a phase II study to assess its effects on patients with NASH. Similarly, a pan-PPAR agonist (α, γ and δ), IVA337, was studied among patients with systemic sclerosis, for its effect on inflammation and fibrosis. A phase II clinical trial (NATIVE) is currently studying IVA337 for its effects on liver histology in patients with NASH.

Thiazolidinediones were not the only group of antidiabetic medications studied among patients with NASH. Glucagon-like peptide-1 (GLP-1) agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors, which are also called incretin-based medications, are used in the management of diabetes. GLP-1 is a gut-derived hormone with a short half-life (about 2 min) and degraded by DPP-4 enzyme in the blood stream. GLP-1 stimulates beta-cell proliferation and differentiation and its actions include lowering blood glucose by inducing insulin secretion and reducing glucagon secretion, as well as suppressing appetite and slowing gastric emptying. DPP-4 inhibitors, as the name implies, inhibit the enzyme DPP-4, thus block the breakdown of GLP-1. Both GLP-1 agonists and DPP-4 inhibitors were assessed for their effect on liver histology. In a study by Armstrong and colleagues, the effect of liraglutide was studied over a 48-week treatment period with pre- and postintervention liver histology assessment. That study demonstrated that 1.8 mg daily liraglutide achieved resolution of NASH in almost 40% of the participants. Similarly, exenatide treatment for 28 weeks resulted in significant improvement in liver histology in another study. Semaglutide is another GLP-1 agonist used in the treatment of diabetes. A phase II clinical trial is currently ongoing to investigate the efficacy and safety of three dose levels (0.1 mg, 0.2 mg and 0.3 mg daily) of semaglutide versus placebo in patients with NASH.

Finally, another target in the management of NASH might be the farnesoid-X-receptor pathway, the receptors of which are highly expressed in liver, kidneys and intestines. Obeticholic acid is
a ligand for farnesoid X receptor, and use has recently been started among patients with primary biliary cholangitis. Given its effects on increasing glucose-stimulated insulin release, augmenting peripheral glucose uptake, inhibiting hepatic lipid synthesis and inducing lipid uptake by adipose tissue, obeticholic acid could be used for the management of NASH. In fact, the FLINT trial compared obeticholic acid with placebo, over 72-week period, and demonstrated that patients who received obeticholic acid for 72 weeks had significant histological improvements compared with patients who received placebo. Those histological improvements included hepatocellular ballooning, lobular inflammation and the severity of fibrosis. In the light of these findings, a larger, phase III clinical trial (REGENERATE) is currently ongoing to evaluate long-term effects of obeticholic acid on fibrosis stage, among biopsy-proven NASH patients.

An important molecule in hepatic lipid metabolism is fibroblast growth factor 19 (FGF19), which is expressed in the intestine and works as an enterohepatic hormone. FGF19 downregulates hepatic expression of the CYP7a1 enzyme and decreases bile-acid synthesis. In this context, NGM282 is a recombinant FGF19 agonist that blocks CYP7a1, decreases bile acid synthesis and improves insulin sensitization. A recent phase II clinical trial demonstrated that NGM282 had acceptable safety among patients with NASH and produced rapid and significant reductions in hepatic fat content. In a similar fashion, FGF21, which is mostly produced in the liver, improves insulin sensitivity. An FGF21 agonist, BMS986036, primarily improves glycemic control by decreasing hepatic glucose production, increasing peripheral glucose turnover and correcting dyslipidemia by increasing high-density lipoprotein cholesterol level. Another agent, BMS986036 is currently being studied in a phase II clinical trial to assess its effect among patients with NASH.

Recently, one of the many areas research has focused on is the enterohepatic circulation of bile acids and cholesterol homeostasis. At this point, bile-acid transporters play a crucial role and comprise multiple members including apical sodium-dependent bile-acid transporter and sodium-taurocholate cotransporting polypeptide. Studies have demonstrated that inhibition of apical sodium-dependent bile-acid transporter results in lowering cholesterol levels and improving insulin sensitization. Volixibat, an apical sodium-dependent bile-acid transporter inhibitor, was shown as safe and tolerable among overweight and obese healthy individuals. In fact, volixibat was selected for fast track by the US Food and Drug Administration and a phase II clinical trial was underway to detect its efficacy among patients with NASH, with a primary endpoint of improvement in NAFLD activity score without worsening of fibrosis. However, just recently, the phase II trial for volixibat was discontinued, without any further explanation for the possible causes.

Another way to downregulate hepatic steatosis is by inhibiting fatty acid synthesis in hepatocytes. During de novo lipogenesis, the rate-limiting step is the conversion of acetyl-coenzyme A to malonyl-coenzyme A, by the enzyme acetyl-coenzyme A carboxylase. Because of this, blocking acetyl-coenzyme A carboxylase will affect the de novo lipogenesis in the hepatocyte. GS-0976 is an acetyl-coenzyme-A-carboxylase inhibitor suggested to be useful in the management of NASH. A recent phase II clinical trial among patients with NASH demonstrated that 12 weeks of 20 mg GS-0976 was able to reduce not only hepatic steatosis detected in MRI, but also markers of fibrosis, such as liver stiffness on MRE, or serum levels of tissue inhibitor of metalloproteinase 1. In another clinical trial, the combination of GS-0976 and GS-9674, which is a farnesoid X-receptor agonist, is being studied for its safety and tolerability in patients with NASH.

In addition to these pathways, other potential pathogenic mechanisms are being targeted. Thereby, previous studies have shown that NAFLD and NASH were associated with liver-specific hypothyroidism resulting in increased triglyceride and cholesterol levels. The activation of thyroid hormone receptor β can decrease hepatic triglyceride levels, thus it was proposed that this system can be used in the treatment of NAFLD and NASH. MGL-3196 is a thyroid hormone receptor β agonist and currently being studied in a phase II clinical trial to investigate its effects on hepatic fat change.

Finally, targeting appetite and the central nervous system has been considered. JKB-121, which is an opioid receptor antagonist, also known as nalmefene, is being studied in a phase II clinical trial for its effects in patients with NASH.
Conclusion
NAFLD and NASH are increasingly being recognized as important causes of cirrhosis and its complications. In this context, presence of multiple components of metabolic syndrome and advanced fibrosis are considered risk factors for liver-related mortality. Although weight loss through lifestyle modification can be effective and is always recommended, the efficacy is limited and not sustained. Although very few drug treatments are available for NASH, a large number of clinical trials of pharmacological agents which target one or more pathways in the pathogenesis of NASH are currently being undertaken. Given the complexity of the pathogenesis of NASH, it is likely that the treatment option may require a combination of different drugs. Additionally, the duration of treatment may require long-term maintenance. In this context, one could envision a short term, intense ‘induction’ treatment regimen, followed by a long term, potentially lifelong maintenance regimen. As we develop these regimens, it is not only to establish their efficacy and safety, but also to show improvement of patient reported outcomes. Finally, these regimens must be cost effective and provide long-term value to patients and society.

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Conflict of interest statement
Dr Z Younossi is a consultant to BMS, Gilead, AbbVie, Intercept, and GSK. All other authors have no conflict of interest to disclose. All authors read and approved the final manuscript.

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