Emerging advances in the pharmacologic treatment of nonalcoholic steatohepatitis and related cirrhosis

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Abstract

The prevalence of nonalcoholic fatty liver disease (NAFLD) is rapidly growing throughout the world. Nonalcoholic steatohepatitis (NASH), the progressive form of NAFLD, is likely to become the leading cause of cirrhosis and etiology for liver transplantation in future decades in the Western World. Most patients with NAFLD have some components of metabolic syndrome, including obesity, insulin resistance, dyslipidemia, and hypertension. NAFLD encompasses a wide spectrum of liver damage, ranging from simple steatosis to NASH, that can progress to advanced liver disease, as well as hepatocellular carcinoma. Unfortunately, the options for the pharmacological treatment of NAFLD are still very limited. Nonetheless, several classes of therapies have shown promise, and are currently being evaluated in large phase 2b and phase 3 trials, creating some hope that selected agents will be approved in the coming years. As NASH is a heterogeneous disease, multiple mechanistic pathways are being targeted to achieve optimal treatment response. Combination therapy is also on the horizon, where 2 or more drugs targeting different mechanistic pathways are being used to boost the clinical response. In this review, we first present the current concept of the pathophysiology of NASH, focusing on the pathways currently targeted in clinical trials. We then present the pharmacological agents that are being evaluated in phase Ib of clinical development and beyond, using histological outcomes, and finally we present preliminary results from the combination trials that have already been initiated.

Keywords Nonalcoholic steatohepatitis, steatosis, fibrosis, pharmacological treatment

Introduction

The prevalence of nonalcoholic fatty liver disease (NAFLD) is rapidly growing and it is currently present in approximately 25% of the global population [1]. Nonalcoholic steatohepatitis (NASH), the progressive form of NAFLD, is one of the top indications for liver transplantation in the United States [2]. On the other hand, the global obesity epidemic is also on the rise, as well as obesity-related complications (NAFLD, cardiovascular disease, obstructive sleep apnea) [3-5]. Most patients with NAFLD have components of metabolic syndrome (MS), including obesity, insulin resistance, dyslipidemia, and hypertension. Cardiovascular mortality is the most common cause of death among patients with NAFLD and NASH [1,6,7]. Furthermore, patients with type 2 diabetes mellitus (T2DM) are at the greatest risk for NAFLD and NASH, with a global prevalence rate of 58% and 65%, respectively [3]. However, NAFLD and NASH can also occur in the absence of obesity in about 10-20% of Americans and Europeans. In Asian populations the prevalence of NAFLD in non-obese patients varies significantly across different countries and is estimated to range from 4-75% (4% in Taiwan, 22% in Japan, 21% in China, 30% in Korea and 75% in India) [8].

NAFLD encompasses a wide spectrum of liver damage, ranging from simple steatosis (or nonalcoholic fatty liver) to NASH. NASH can develop into advanced fibrosis and cirrhosis, as well as hepatocellular carcinoma [9]. Based on histological data, 21% of NAFLD patients in the USA have NASH [1,8]. The prevalence of comorbid conditions associated with NASH is 82% for obesity, 48% for T2DM, 82% for hyperlipidemia, 76% for metabolic syndrome, and 70% for arterial hypertension [8].
Of all aspects of NAFLD, the slowest advances have occurred in the therapeutic field. Currently, there is no approved drug for the treatment of NASH. However, in the last 40 years there has been progress in understanding the pathophysiology, diagnosis, epidemiology and even natural history of NASH [10]. Several ongoing clinical trials are currently testing new molecules for NASH therapy [11]. Clinical research is likely to identify new targets for therapy and may further explore combination therapy to target synergistic pathways. Additionally, the drugs that have failed in phase III trials (for example elafibranor, cenicriviroc, and selonsertib) contribute to the trajectory of further research. The main challenges in the design of clinical trials on NASH include the complex pathophysiology of the disease, the heterogeneous population enrolled in the studies and the high degree of variability in assessing histological endpoints.

In this review, we first present the current concept of the pathophysiology of NASH, focusing on the pathways currently targeted in clinical trials. We then present the pharmacological agents that are being evaluated in phase III and phase IIb of clinical development, and finally touch upon the combination trials that have already been initiated.

**NASH pathophysiology**

The pathogenesis of NAFLD is multifactorial. Therefore, several pathogenetic pathways must be evaluated. The cornerstone of the pathogenesis is based on the concept that, under certain circumstances, fat accumulation can result in lipotoxicity and subsequent immune system activation. Insulin resistance, another key pathogenic driver of NAFLD, is a shared characteristic of T2DM and obesity that may also contribute to the increased levels of free fatty acids (FFAs) and carbohydrates seen in patients with NASH [12]. Manipulation of fatty acid metabolism through different pathogenetic pathways may contribute to the inhibition of the natural course of NAFLD [13]. Finally, genetic susceptibility, alcohol consumption and dysbiosis may also play a role in NAFLD progression and NASH development [12]. The main mechanisms that contribute to the disease spectrum of NAFLD, along with the pharmacological agents that target them, are summarized in Fig. 1 and presented in more detail below.

**Hepatic steatosis, lipotoxicity and glucotoxicity**

Fatty liver begins with the hepatic accumulation of lipid droplets in the cytoplasm of hepatocytes. This is a result of the imbalanced metabolism of lipids. The influx and synthesis of lipids exceeds the capacity of the hepatocyte to secrete or utilize the excess lipid droplets [12]. Hepatic steatosis may be isolated, or may progress to an inflammatory process of hepatocyte ballooning and lobular inflammation [13]. Multiple types of lipids are involved in the development of hepatic lipotoxicity. Lipids with increased levels in patients with NASH are FFAs, triglycerides (TGs), free cholesterol, lysophosphatidylcholine (LPC), bile acids, and ceramides. However, not all lipids are harmful. The main drivers of liver cell injury appear to be the lipotoxicity caused by FFAs and their derivatives combined with mitochondrial dysfunction [14].

Hepatic nonesterified fatty acids (NEFA) play an important role in the pathogenesis of NASH. There are 3 major sources of NEFA. Approximately 60% are from insulin signaling. Insulin signaling increases adipose TG lipolysis, which increases hepatic fatty acid delivery. Approximately 26% of NEFA are from the conversion of carbohydrates within the liver (de novo lipogenesis [DNL]), a process where glucose and fructose are enzymatically converted to lipids. Lastly, approximately 14% of NEFA are due to excess dietary intake [10,12]. NEFA are stored in the liver as TG-rich lipid droplets, leading to hepatic steatosis, or exported into the circulation as very low-density lipoprotein to adipose tissue [10].

Studies have shown that palmitic acid (a saturated fatty acid) can activate peroxisome proliferator-activated receptor α (PPARα). Furthermore, elevated levels of ceramides, free cholesterol and palmitic acid can cause impairment in insulin signaling, mitochondrial dysfunction and inflammation (elevated levels of proinflammatory cytokines and production of reactive oxygen species) resulting in apoptosis and cell death [12]. Hepatic glucotoxicity is the toxic effect on the liver cells and tissues of excess carbohydrate intake and hyperglycemia. Continuous hyperglycemia causes low-grade inflammation, which contributes to insulin resistance [14]. Finally, dietary fructose has been shown to be highly lipogenic in humans [15].

Therapeutic strategies with antisteatotic effects are mediated through targeting the increase in fatty acid oxidation (PPAR α/δ agonists; fibroblast growth factor [FGF] 21 agonists; thyromimetics), the inhibition of DNL (acyl-coenzyme A [CoA] carboxylase inhibitor), the increase in fatty acid desaturation (stearoyl-CoA desaturase inhibitors) or by improving insulin resistance (PPARγ and glucagon-like peptide 1 [GLP-1] agonists) [11].

**Cell stress and apoptosis**

Studies have shown that NASH is characterized by an elevation in total mitochondrial mass, with a 30-40% lower respiration maximum, associated with mitochondrial uncoupling and leaking [16]. Both genetic and epigenetic factors are thought to contribute to mitochondrial dysfunction in NASH [17]. Once FFA flux is increased, the mitochondria become exhausted and FFAs...
are handled in other sites, including peroxisomes (β-oxidation) and the endoplasmic reticulum (ER) (oxygenation), leading to oxidative stress and cell death [18]. The ER is a major site for calcium storage, carbohydrate metabolism, protein synthesis and folding, and lipid and steroid synthesis [19]. Any imbalance in these processes or saturation of the ER membrane with lipids can lead to ER stress, apoptosis and cell death [20].

Inflammation

Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD-like) receptors (or NLRs) and recognition signal receptors are involved in innate immune system activation and play a role in the development of NASH [21]. Their activation leads to a proinflammatory cytokine cascade, which induces insulin resistance and fatty liver development [22, 23]. In NASH, intestinal permeability increases and the intestine-derived pathogen-associated molecular patterns and lipopolysaccharides (LPS) translocate to the liver and lead to activation of TLRs [24]. This activation releases several cytokines, including tumor growth factor-β, interleukin-1β and tumor necrosis factor-α, which leads to lipid accumulation, the activation of hepatic stellate cells (HSCs), and hepatic apoptosis [25, 26].

Fibrosis

Liver fibrosis is the accumulation of an excessive amount of extracellular matrix proteins (mainly type I collagen). Fibrosis is mainly driven by myofibroblasts, HSCs, portal fibroblasts and mesothelial cells. Inflammatory cells, such as macrophages, NK cells, and lymphocytes, cause the activation of HSCs, resulting in the development of fibrosis and cirrhosis [27]. Notably, all phase III studies in patients with NASH using pharmacological agents with primarily antifibrotic effects (cenicriviroc, emricasan, selonsertib, simtuzumab) have failed [11].

Genetics

Several studies have identified genes with multiple polymorphisms associated with NAFLD. However, 2 genes (PNPLA3 and TM6SF2) have been identified as risk factors for NAFLD. The rs738409[G] allele of PNPLA3 is associated with higher liver fat content, the necroinflammatory state and liver fibrosis [28]. The other genetic variant rs58542926 allele of TM6SF2 is associated with increased risk for progressive NASH and reduced risk of cardiovascular disease [29].
**Gut-liver axis**

Leaky gut is a condition commonly seen in NASH patients and is associated with translocation of LPS through the portal circulation to the liver [30]. Circulating endotoxins have been found to be elevated in NASH patients and chemokines may play a role in regulating gut permeability [31,32]. Gut microbiota also play a role in the pathogenesis of NASH [31,33]. Several mechanisms are involved in dysbiosis: alterations in gut permeability linked to dysregulation of epithelial tight junction formation; defective inflammosome sensing and disrupted inflammatory responses; altered choline metabolism by the microbiota; bacterial metabolites produced, degraded, or modulated in the gut, including LPS, short chain fatty acids and bile acids; and increased delivery to the liver of ethanol produced by the gut microbiota [28,33].

**Nuclear receptors**

Nuclear receptors regulate glucose and lipid metabolism in the liver. They consist of 7 families known as NR0-NR6. NR1 plays an important role in NAFLD. NR1 includes NR1C1-3 (PPAR	extsubscript{0}, NR1H2-3 (the liver X receptors [LXR]), NR1H4 (the farnesoid X receptor [FXR]), NR1I2 (the constitutive androstane receptor [CAR]), and NR1I3 (the pregnane X receptor [PXR]) [34,35]. These receptors may be potential therapeutic targets in NASH. Ongoing clinical trials are actively investigating FXR and PPAR.

Therapeutic trials in NASH primarily focus on histological improvement in inflammation and/or fibrosis. Subsequently, one-stage improvement in liver fibrosis or resolution of NASH is currently the usual endpoint in NASH clinical trials. However, the regulatory approval pathway for pharmacological therapies for NASH requires therapies to show clinical benefit in improving liver-related outcomes, which constitutes a relevant endpoint in all current phase III studies [36].

**Pharmacological agents in phase III clinical development**

Drugs that have reached phase III of clinical development are by definition closer to regulatory approval. Currently, there are 5 agents that have progressed to this stage and are being evaluated in large, well-designed studies with both histological and long-term clinical outcomes (Table 1). In addition, there are 2 other investigator-initiated phase III studies, smaller in size, evaluating further pharmacological agents. In this section of the review, we summarize the evidence for all of these agents. In Table 2 we present data on other drugs that are being evaluated in phase II studies, either without histological endpoints or in narrow subpopulations [37].

**Aramchol (targeting liver lipid metabolism)**

Aramchol is a synthetic lipid molecule obtained by conjugating 2 natural components, cholic acid (a bile acid) and arachidic acid (a saturated fatty acid) [38]. Aramchol inhibits the liver enzyme stearoyl coenzyme A desaturase 1 (SCD1), a key enzyme that modulates fatty acid metabolism. SCD1 inhibition decreases the synthesis and increases the \( \beta \)-oxidation of fatty acids, thus resulting in decreased hepatic storage of TGs and fatty acid esters. In addition, aramchol activates cholesterol efflux by stimulating the ATP-binding Cassette Transporter A1 (ABCA1), a pan cellular cholesterol export pump, and has shown an antiatherogenic effect in animal studies [39,40].

A proof-of-concept, randomized, double-blind trial using aramchol (100-300 mg/day) or placebo for 3 months in 60 patients with biopsy-confirmed NAFLD (6 with NASH) showed a dose-dependent decrease in hepatic fat according to magnetic resonance spectroscopy (MRS) assessments (significance was only achieved in the 300 mg treatment group) [38]. No serious adverse events were observed in this initial study. Subsequently, a second multicenter, randomized, double-blind, placebo-controlled phase Ib study (the ARREST study) evaluated the efficacy and safety of higher doses of aramchol (400 mg and 600 mg) in NASH patients who presented as overweight or obese, and with pre-diabetes or diabetes (247 patients, 52 weeks, and 13-week follow up) [41]. The primary outcome was the percentage change in intrahepatic TG concentration measured by MRS, while the secondary outcomes included fibrosis improvement for at least one stage without worsening of NASH or NASH resolution without worsening of fibrosis. The study confirmed that a larger number of patients in the aramchol 600 mg arm achieved resolution of NASH without worsening of fibrosis (16.7% vs. 5% for placebo; odds ratio [OR] 4.74, 95% confidence interval [CI] 0.99-22.66) and fibrosis improvement by at least one stage without worsening of NASH (29.5% vs. 17.5% for placebo; OR 1.88, 95%CI 0.7-5.0).

A phase III randomized-controlled study (the ARMOR study; NCT04104321) is currently recruiting 2000 patients at high risk of progression (fibrosis stage F2 or F3) who are also overweight or obese and have either pre-diabetes or diabetes. Patients are randomized to receive 300 mg of aramchol b.i.d. or placebo. Primary outcomes are the effects on liver histology at 52 weeks and the effects on composite long-term outcomes (all-cause mortality, need for transplantation, hospital admission due to hepatic decompensation) at 5 years. In addition, an open-label part has been now added in selected sites that are less affected by the COVID-19 pandemic. In this part of the study, 150 subjects will be randomized into 3 groups according to post-baseline biopsy. The objective of the open-label part is to evaluate the safety and pharmacokinetics of b.i.d. administration of 300 mg of aramchol and to explore the kinetics of histological outcome measures and noninvasive tests associated with NASH and fibrosis for the treatment duration of at least 72 weeks and up to 120 weeks.

**Lanifibranor (targeting PPARs)**

Lanifibranor is a pan-PPAR agonist. PPARs represent a family of transcription factors that consists of 3 different receptors (PPAR\(\alpha\), PPAR\(\beta/\delta\), and PPAR\(\gamma\)) and act as lipid
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Table 1 Therapeutic strategies in phase 3 clinical trials for NASH

| Drug name   | Mechanism of action | Subjects                                      | Study title                                                                 | Dose                                | Duration    |
|-------------|---------------------|-----------------------------------------------|-----------------------------------------------------------------------------|-------------------------------------|-------------|
| Aramchol    | SCD1 inhibition and ABCA1 stimulation | 2000 with NASH, fibrosis stage 2-3, overweight or obese, prediabetes or T2DM | Phase 3, multinational, randomizer, double-blind, placebo-controlled (ARMOR) | 300 mg PO, BID                        | 2019-Ongoing |
| Lanifibranor| PPARs agonist       | 2000 with NASH and fibrosis stage 2-3         | Phase 3, multicenter, randomized, double-blind, placebo-controlled (NATIV3) | 800 mg/day or 1200 mg/day PO         | 2021-Ongoing |
| Obeticholic Acid | FXR agonist    | 2480 with NASH and fibrosis stage 2-3         | Phase 3, multicenter, randomized, double-blind, placebo-controlled (REGENERATE) | 10 mg/day or 25 mg/day PO           | 2015-Ongoing |
|             |                    | 919 with compensated cirrhosis due to NASH   | Phase 3, multicenter, randomized, double-blind, placebo-controlled (REVERSE) | 10 mg/day or 25 mg/day PO           | 2017-Ongoing |
| Lanifibranor|                    | 2000 with NASH and fibrosis stage 2-3         | Phase 3, multicenter, randomized, double-blind, placebo-controlled (NATIV3) | 80 mg/day or 100 mg/day PO          | 2019-Ongoing |
| Resmetirom  | Highly selective agonist of THR-β | 2000 with NASH and fibrosis stage 2-3         | Phase 3, multicenter, randomized, double-blind, placebo-controlled (MAESTRO-NASH) | 80 mg/day or 100 mg/day PO          | 2019-Ongoing |
| Semaglutide | GLP-1 agonist       | 1200 with NASH and fibrosis stage 2-3         | Phase 3, multicenter, randomized, double-blind, placebo-controlled (ESSENCE) | 2.4 mg/week SC                      | 2021-Ongoing |

NASH, nonalcoholic steatohepatitis; T2DM, type 2 diabetes mellitus; SCD1, stearoyl-CoA desaturase 1; ABCA1, ATP-binding cassette transporter A1; PPARs, peroxisome proliferator-activated receptors; PO, per os; BID, bis in die; FXR, farnesoid X receptor; THR-β, thyroid hormone receptor β; GLP-1, glucagon-like peptide; SC, subcutaneously

Table 2 Studies with non-histological endpoints or special indications

| Drug name   | Mechanism of action | Subjects                                      | Study title                                                                 | Dose                                | Duration    |
|-------------|---------------------|-----------------------------------------------|-----------------------------------------------------------------------------|-------------------------------------|-------------|
| Oltipraz    | AMPK-S6K1 and LXRg-SREBP-1c inhibition | 60 with NAFLD, except liver cirrhosis         | Phase 2, multicenter, randomized, double-blind, placebo-controlled         | 30 mg or 60 mg PO, BID              | 2013-Completed |
|             |                    | 144 with NAFLD, except liver cirrhosis       | Phase 3, multicenter, randomized, double-blind, placebo-controlled (NCT04142749) | 90 mg PO, TID                        | 2019-Ongoing |
| Secukinumab | Anti-interleukin 17A | 90 patients with psoriasis and NAFLD         | Phase 3, multicenter, randomized, double-blind (pINPOINT)                   | 300 mg SC/week followed by Q4W up to Week 20 | 2020- Ongoing |

NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; AMPK-S6K1, adenosine monophosphate-activated protein kinase dependent p70 ribosomal S6 kinase-1; LXRg-SREBP-1c, liver X receptor sterol regulatory element-binding protein-1c gene; TID, 3 times a day; Q4W, every 4 weeks; PO, per os; SC, subcutaneously

sensors in various tissues [42]. PPARs play critical roles in the regulation of liver homeostasis, fatty acid oxidation, insulin sensitivity, TG metabolism, and adipogenesis. They prevent fibrogenesis by keeping HSCs in the quiescent phase and regulating the inflammatory response [43].

Lanifibranor recently completed a phase IIb, biopsy-controlled study (the NATIVE study) in 247 patients with NASH receiving either 800 or 1200 mg/day of active drug vs. placebo for 6 months. The primary endpoint was a 2-point reduction in the activity part of the SAF score (combining inflammation and ballooning) without worsening of fibrosis; the key secondary endpoints were resolution of NASH without worsening of fibrosis and improvement of fibrosis without worsening of NASH [44]. The results showed that the primary endpoint was met with the 1200 mg dose (55% vs. 33%, P=0.007), but favored both doses (1200 and 800 mg) over placebo for resolution of NASH without worsening of fibrosis (49% and 39%, respectively, vs. 22%), improvement in fibrosis stage of at least 1 without worsening of NASH (48% and 34%, respectively, vs. 29%), and resolution of NASH plus improvement in fibrosis.
stage of at least 1 (35% and 25%, respectively, vs. 9%) [45]. The dropout rate for adverse events in this trial was less than 5% and was similar across the trial groups. However, diarrhea, nausea, peripheral edema, anemia, and weight gain occurred more frequently with lanifibranor than with placebo.

The company has designed a phase III randomized-controlled study (the NATIV3 study; NCT04849728) that plans to recruit 2000 patients with NASH and fibrosis stage F2 or F3. Patients will be randomized to receive lanifibranor (800 mg/day), lanifibranor (1200 mg/day), or placebo, employing a 1:1:1 randomization scheme. Primary outcomes are the effects of lanifibranor compared to placebo on: i) NASH resolution and improvement of fibrosis assessed by liver histology at 72 weeks; and ii) delaying NASH disease progression measured by a composite endpoint that includes progression to cirrhosis, liver-related clinical outcome events, or all-cause death.

**Obeticholic acid ([OCA] targeting bile acid receptors)**

OCA represents the first-in-class selective FXR agonist, originally described for its anticholestatic properties. OCA is produced with the addition of an ethyl group to chenodeoxycholic acid, the natural FXR agonist in humans [46]. The FXR belongs to the nuclear receptor superfamily mainly expressed in the liver and intestine. It regulates a wide variety of target genes involved in the control of bile acids and lipids and the homeostasis of glucose [45]. The FXR activation induces several effects, including the decreased expression of enzymes involved in de novo lipogenesis and the release of FGF 19 from the intestine, which produces additional metabolic effects.

A phase IIb clinical trial of OCA (the FLINT trial) (25 mg/day of oral OCA vs. placebo for 72 weeks) was terminated after a pre-planned interim analysis at 24 weeks because of overt histological efficacy (≥2 points decrease in NAS, without worsening of fibrosis) [47]. Fifty (45%) of 110 patients in the OCA group meant to have biopsies at baseline and 72 weeks had improved liver histology, compared with 23 (21%) of 109 such patients in the placebo group (relative risk [RR] 1.9, 95%CI 1.3-2.8; P=0.0002).

OCA is currently being evaluated in a phase III trial (the REGENERATE trial; NCT02548351) at doses of 10 and 25 mg/day vs. placebo in NASH patients with fibrosis; liver biopsies were scheduled at screening, at 18 and 48 months, and at the end of the study. The results of the interim 18-month analysis in 931 patients with fibrosis stage F2-F3 showed improvement in fibrosis: 12% in the placebo group, 18% in the 10 mg OCA group, and 23% in the 25 mg OCA group (P=0.0002) [48]. The NASH resolution endpoint was not met in the intention-to-treat population (8%, 11%, and 12%, respectively). However, a post hoc analysis showed that approximately twice as many patients treated with 25 mg OCA achieved NASH resolution compared with placebo in both intention-to-treat (23% vs. 12%; RR 1.9, 95%CI 1.4-2.8) and per-protocol analyses (29% vs. 16%; RR 2.2, 95%CI 1.4-3.2). A dossier was submitted to the US Food and Drug Administration for regulatory approval on the basis of more than 1700 patients treated with obeticholic acid, but the agency required additional efficacy and safety data to support accelerated approval, while the long-term phase continues [49].

In terms of adverse events, OCA has been mainly associated with dose-dependent pruritus and increased low density lipoprotein (LDL) cholesterol. In the REGENERATE trial, OCA at the dose of 25 mg daily was associated with pruritus in half of the patients, with severe intensity in 28% [48]. Furthermore, elevation in LDL cholesterol level requiring addition of a statin was noted twice as often as in the placebo group. Finally, OCA at the dose of 25 mg was also associated with a greater percentage of hepatobiliary events in the form of gallstones or cholecystitis (3%), compared to patients receiving placebo (<1%). These safety issues have raised concerns about the long-term tolerability, cardiovascular morbidity and gallstone-related events; thus, additional safety data from the long-term phase of the study are eagerly awaited before the final evaluation of this agent.

**Resmetirom (targeting liver specific thyroid hormone receptors [THRβ])**

Resmetirom is an oral, highly selective agonist of THR-β specifically acting in the liver without any systemic effects (mediated through THR-α in the heart and bone) [50]. THR-β is responsible for regulating hepatic lipid metabolism often impaired in NAFLD, making NAFLD a condition of “hepatic hypothyroidism”. The mechanism by which resmetirom reduces hepatic fat in NASH is probably dependent on the restoration of normal mitochondrial function and increased β oxidation.

Resmetirom was initially tested in a phase II quadruple blind, randomized-controlled trial in 125 participants with at least 10% liver fat content at Magnetic Resonance Imaging Proton Density Fat Fraction (MRI-PDFF) and biopsy proven NASH (fibrosis F1-F3 and disease activity), of whom 84 received resmetirom and 41 placebo [51]. Compared with placebo, resmetirom significantly reduced MRI-PDFF from baseline, both after 12 weeks (-36.3% resmetirom vs. -9.6% placebo, P<0.0001) and after 36 weeks (-37.3% resmetirom vs. -8.9% placebo, P<0.0001). Based on liver biopsy, resmetirom achieved NASH resolution in a higher percentage than placebo (27.4% vs. 6.5%, P=0.02), although the percentage of patients who achieved at least one point in fibrosis improvement did not differ between the 2 treatment groups. Moreover, resmetirom was associated with a significant decrease in the levels of serum lipids compared to placebo. Resmetirom was generally well tolerated. The most common adverse events were diarrhea and nausea.

Two phase III trials of resmetirom, MAESTRO-NASH and MAESTRO-NAFLD1, are currently recruiting patients. MAESTRO-NASH (NCT03900429) is estimated to be completed in 2024. It will include 2000 adults with biopsy-proven, non-cirrhotic NASH and fibrosis and will test resmetirom at a dose of 80 mg or 100 mg daily compared to placebo. In addition, the trial will evaluate the effect of resmetirom on a composite long-term outcome measured by the number of patients with new onset of cirrhosis, liver-related
clinical outcomes and all-cause mortality up to 54 months. MAESTRO-NAFLD1 (NCT04197479) has recently started and will include 700 adults with MRI-PDFF liver fat fraction 8% or greater and suspected NASH, randomized into 4 arms: open label, placebo (double blind), resmetirom 80 mg (double blind), and resmetirom 100 mg (double blind). The primary outcome of this non-biopsy study is the incidence of adverse events after 52 weeks of treatment.

### Semaglutide (targeting GLP-1)

Semaglutide is a human GLP-1 receptor agonist (RA) with increased homology (94%) to human GLP-1 [52]. GLP-1 is an intestinal hormone, released from L cells in the small intestine in response to meals, that has multiple metabolic effects: it stimulates insulin secretion and inhibits glucagon secretion, increases energy disposal, delays gastric emptying and improves satiety [53]. GLP-1RA are commonly used to treat diabetes and several studies reported a significant reduction of liver fat in response to treatment [54]. Specifically, semaglutide was associated with reduced levels of alanine aminotransferase, markers of inflammation and reduced cardiovascular risk among patients with type 2 diabetes mellitus at high cardiovascular risk [55,56].

A 72-week randomized, double-blind, multicenter phase II study of semaglutide in patients with biopsy-confirmed NASH and liver fibrosis of stage F1-F3 was published very recently [57]. After 72 weeks of therapy with the highest dosage tested (0.4 mg q.d., s.c.), 33/56 (59%) patients with fibrosis F2-F3 met the usual primary endpoint of NASH resolution without worsening of fibrosis, compared with 10/58 (17%) patients in the control arm. Among patients taking the 0.1 mg and 0.2 mg doses, 40% and 36% achieved the endpoint, respectively. However, the confirmatory secondary endpoint of fibrosis improvement without worsening of NASH was not met. Fibrosis improved by one stage in all arms, with no difference between placebo (33%) and the 0.4 mg semaglutide group (43%). Among patients taking the 0.1 mg and 0.2 mg doses, 40% and 36% achieved the endpoint, respectively. Moreover, semaglutide was associated with significant weight loss. In the 0.4 mg semaglutide group, the mean percent change in body weight was -13%. Gastroenterological disorders (nausea, constipation, decreased appetite, vomiting, and abdominal pain) were the most common reported adverse events. The incidence of nausea, constipation, and vomiting was higher in the 0.4 mg group than in the placebo group (nausea, 42% vs. 11%; constipation, 22% vs. 12%; and vomiting, 15% vs. 2%). Serious gastrointestinal disorders were also more common in the semaglutide groups (2-5%) than in the placebo group (0%), and were the most common reason for discontinuation among patients who received semaglutide (4% of the patients). Malignant neoplasms were reported in 3 patients who received semaglutide (1%) and in no patients who received placebo. Overall, neoplasms (benign, malignant, or unspecified) were reported in 15% of the patients in the semaglutide groups and in 8% in the placebo group; no pattern of occurrence in specific organs was observed.

Currently, semaglutide is being evaluated at a dose of 2.4 mg once-weekly in a phase III clinical trial that will recruit 1200 patients (the ESSENCE trial; NCT04822181). The trial will last for 5 years and is scheduled, in agreement with all the other phase III studies, to assess both resolution of NASH and fibrosis improvement and the time to first liver-related clinical event (histological progression to cirrhosis, all-cause mortality, liver-induced model for end-stage liver disease score greater than or equal to 15, liver transplant or hepatic decompensation events).

### Dapagliflozin (targeting glucose resorption)

Dapagliflozin belongs to the family of gliflozins, which act by inhibiting sodium-glucose co-transporter-2 (SGLT-2). SGLT-2 inhibitors block glucose resorption from the proximal tubule and promote glycosuria, calorie waste and weight loss [58]. This possibly translates into reduced lipid burden to the liver. Most approved gliflozins have been tested for their effects on biomarkers of liver steatosis and fibrosis, but very few histological data are available [59,60]. A recent meta-analysis of non-biopsy randomized controlled studies confirmed that SGLT-2 inhibitors seem to be a promising treatment option for NAFLD [61].

Dapagliflozin is currently being tested in China in a randomized, placebo-controlled study (the DEAN study) that is plans to assess the efficacy and safety of dapagliflozin in improving NASH, as determined by liver biopsies and metabolic risk factors (NCT03723252). The trial will recruit 100 patients who will receive oral dapagliflozin at 10 mg q.d., or matching placebo. The primary outcome will be the scored liver histological improvement over 12 months.

### Saroglitazar (targeting PPARs)

Saroglitazar is a dual (PPARα/γ) agonist. As discussed earlier, PPARs play critical roles in the regulation of liver homeostasis, lipid metabolism and insulin sensitivity. In a phase II study (the EVIDENCES IV study), presented in abstract form, 106 patients were randomly assigned to placebo or saroglitazar at 1 mg, 2 mg or 4 mg daily doses [62]. Saroglitazar was associated with alanine aminotransferase (ALT) reduction after 16 weeks of treatment. In addition, the drug at the dose of 4 mg led to an absolute decrease in liver fat as assessed by MRI-PDFF (-4.21% vs. -0.31%, P=0.01).

Saroglitazar is currently being tested in India in a randomized, 4-arm study that will evaluate the efficacy and safety of saroglitazar, vitamin E and lifestyle modification in patients with NASH (NCT04193982). The trial will recruit 250 patients who will receive saroglitazar 4 mg daily, vitamin E 400 IU b.i.d. or the combination of saroglitazar and vitamin E, or will follow advice on lifestyle changes, including targeting 7-10% weight loss in the 6-month period of the study. The primary outcome of the study is the change in NAFLD fibrosis score at weeks 8, 16 and 24. Secondary outcomes will include changes in fibrosis on liver biopsy.
Pharmacological agents in phase IIb clinical development

In this section, we present drugs that are being evaluated in phase IIb of clinical development using liver biopsy or hepatic venous pressure gradient (HVPG) to determine the primary outcome. Most drugs that will complete this phase showing efficacy with an accepted safety profile will move to phase III after acceptance from the regulatory agencies. The field of phase II trials includes many additional drugs (mainly evaluated in non-biopsy studies), the presentation of which is beyond the scope of this review. However, they are summarized in Table 3 in order to offer the reader the chance to become familiar with the complete armamentarium of the agents that are currently evaluated in NASH.

Agents targeting fibroblast growth factors, FGF (FGF analogs)

FGF play diverse roles in the metabolic homeostasis, affecting bile acid, glucose and lipid metabolism [63]. FGF analogs, the most advanced being FGF19 and FGF21 analogs, are currently being vigorously evaluated for the treatment of NASH in phase II trials. These agents are able to stimulate adiponectin secretion, thus reducing insulin resistance and inflammation, as well as to reduce body weight. FGF analogs that have already been evaluated, or are scheduled to be evaluated in trials with histological endpoints, are aldafermin (an engineered version of FGF19), pegbelfermin (a pegylated FGF21 analog) and efruxifermin (another engineered, FGF21 compound, which is actually a human immunoglobulin [IgG1] Fc-FGF21 fusion protein).

In a phase II, 24-week study in 78 NASH patients with fibrosis F2-F3, aldafermin given daily as a s.c. injection met the primary endpoint of significant reduction in liver fat vs. placebo [64]. At the histological level, there was a trend, but no significant differences, toward improvement in fibrosis of more than one stage (38% vs. 18%), as well as NASH resolution with no worsening of fibrosis (24% vs. 9%). In this study, LDL cholesterol levels were increased at week 2 in patients receiving aldafermin (mean change from baseline of 44 mg/dL in the aldafermin group, and -1 mg/dL in the placebo group). This adverse event was effectively managed with rosvastatin (at week 24, 36% of patients in the placebo group and 96% in the aldafermin group were taking rosvastatin). Partial results from the phase IIb randomized, double-blind, placebo-controlled study of the drug (the ALPINE 2/3 study) evaluating aldafermin at doses of 0.3 mg, 1 mg and 3 mg have been previously presented [65]. The complete results will be presented at the 2021 Liver Meeting of the American Association for the Study of Liver Diseases (AASLD). However, the company has already announced that the study did not meet the primary endpoint of fibrosis improvement by >1 stage with no worsening of NASH, although certain secondary endpoints, including NASH resolution, were achieved. Subsequently, the company announced that it does not plan to pursue phase III clinical development of aldafermin in F2/F3 NASH. Notably, a study (the ALPINE4 study) of aldafermin in NASH patients with cirrhosis is continuing to recruit subjects (NCT04210245).

Pegbelfermin, given s.c. q.d., was initially tested vs. placebo in a multidose, 16-week, phase II trial. The trial was terminated early, because of overt superiority of the study drug regarding the absolute change in hepatic fat content (MRI-PDFF) [66]. Specifically, a significant decrease in absolute hepatic fat fraction was noted in the group receiving 10 mg pegbelfermin daily (-6.8% vs. -1.3%; P=0.0004) and in the group receiving 20 mg pegbelfermin weekly (-5.2% vs. -1.3%; P=0.008), compared with the placebo group. On this basis, the drug was moved to phase IIb in patients with stage 3 liver fibrosis (the FALCON1 study; NCT03486899) and NASH cirrhosis (the FALCON2 study; NCT03486912).

Finally, efruxifermin has been investigated in a 16-week, phase II study across the whole spectrum of fibrosis stages (the BALANCED study; NCT03976401). The primary endpoint was change in steatosis on MRI-PDFF at 12 weeks. Patients who met the primary endpoint (50/80; only 2 among controls) were eligible for biopsy at 16 weeks, which showed improvement in fibrosis without NASH worsening in 48% of cases, with 28% achieving improvement by at least 2 stages [67]. Subsequently, the drug, at a weekly dose of 28 mg and 50 mg, has moved to phase IIb evaluation in a randomized, double-blind, placebo-controlled study (NCT04767529).

Agents targeting galectins (Galectin-3 inhibitor, Belapectin)

Galectins are a group of cytosolic proteins that are markedly increased in inflammation and fibrosis. Galectin-3, the most prominent galectin, is secreted specifically by macrophages and upon tissue injury can be activated and contribute to the mechanisms that induce hepatic fibrosis. Galectin inhibitors are a new class of drugs that target galectins and disrupt their functions [68]. Chalassani et al performed a phase IIb randomized, double-blind study of the galectin-3 inhibitor belapectin in adults with cirrhosis and portal hypertension secondary to NASH [69]. After biweekly infusions of 2 or 8 mg/kg belapectin vs. placebo for 52 weeks (n=54 in each arm), they found no significant differences in the primary outcome of reduction in HVPG. However, in subgroup analysis, patients without varices receiving 2 mg/kg belapectin vs. placebo for 52 weeks (n=54 in each arm), they found no significant differences in the primary outcome of reduction in HVPG. However, in subgroup analysis, patients without varices receiving 2 mg/kg belapectin (n=81) experienced a reduction in HVPG (-1.61 mmHg) compared with placebo (P=0.02). Adverse events were similar across all arms and there were no differences in complications of cirrhosis between groups. Based on the findings from the phase IIb trial, the drug is currently being evaluated in an adaptive, 2-stage phase II/III trial in patients with NASH cirrhosis without esophageal varices at baseline (the NAVIGATE trial; NCT04365868). The primary endpoint is the proportion of patients in the belapectin treatment groups who develop new esophageal varices at 7 weeks of treatment compared to placebo.

Agents targeting THR (THR-β agonist, VK-2809)

As discussed earlier, THR-β is responsible for regulating hepatic lipid metabolism. The results of a phase Ia study
assessing VK-2809, a THR-β agonist, at a daily dose of 5 mg or 10 mg on alternate days, or placebo, showed a significant reduction in both LDL cholesterol (primary outcome) and liver fat in all active treatment arms [70]. The drug was safe and

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**Table 3 Therapeutic strategies in phase 2 clinical trials for NAFLD and/or NASH**

| Drug name        | Mechanism of action | Subjects                                                                 | Study title                                                                 | Dose                  | Enrolment period |
|------------------|---------------------|--------------------------------------------------------------------------|------------------------------------------------------------------------------|-----------------------|------------------|
| Pegbelfermin     | FGF21-receptor agonist | 155 with NASH and compensated liver cirrhosis                           | Phase 2b, randomized, double-blind, placebo-controlled (FALCON 2)            | 10, 20 or 40 mg/week SC | 2018-Ongoing     |
|                  |                     | 160 with NASH and fibrosis stage 3                                       | Phase 2b, randomized, double-blind, placebo-controlled (FALCON 1)            | 10, 20 or 40 mg/week SC | 2018-Ongoing     |
| Efruxifermin     | Fc-FGF21 agonist    | 110 with NASH and fibrosis stage 1-4                                     | Phase 2a, randomized, double-blind, placebo-controlled                      | 28, 50 or 70 mg/week SC | 2019-Ongoing     |
| Firsocostat      | ACC inhibitor       | 127 with NASH and fibrosis                                               | Phase 2, randomized, double-blind, placebo-controlled                      | 5, 10 or 20 mg/day PO  | 2016-Completed   |
| PF-05221304      | ACC inhibitor       | 305 with NASH                                                            | Phase 2a, randomized, double-blind, placebo-controlled, dose-ranging, parallel group | 2, 10, 25 or 50 mg/day PO | 2019-Completed   |
| Liraglutide      | GLP-1 agonist       | 52 with NASH                                                             | Phase 2, randomized, double-blind, placebo-controlled (LEAN)                | 1.8 mg/day SC         | 2014-Completed   |
| Tirzepatide      | GIP and GLP-1 agonist | 196 with NASH and fibrosis stage 2-3                                     | Phase 2, randomized, double-blind, placebo-controlled (SYNERGY-NASH)        | 5, 10 or 15 mg/week SC | 2019-Ongoing     |
| Seladelpar       | PPAR δ agonist      | 181 with NASH and fibrosis stage 1-3                                     | Phase 2, randomized, double-blind, placebo-controlled                      | 10, 20 or 50 mg/day PO | 2018-Ongoing     |
| Saroglitazar     | PPAR α/γ agonist    | 240 with NASH and fibrosis stage 2-3                                     | Phase 2b, multicenter, prospective, randomized, double-blind, placebo-controlled | 2 or 4 mg/day PO      | 2021-Ongoing     |
|                  |                     | 106 with NAFLD and/or NASH                                               | Phase 2b, multicenter, prospective, randomized, double-blind, placebo-controlled | 1, 2 or 4 mg/day PO    | 2017-Completed   |
|                  |                     | 16 with NASH and fibrosis stage 0-3                                     | Phase 2, prospective, randomized, double-blind, placebo controlled         | 2 or 4 mg/day PO       | 2019-Completed   |
| Tropifexor       | FXR agonist         | 351 with NASH and fibrosis stage 1-3 (Part A, B) or fibrosis stage 2-3 (Part C) | Phase 2, multicenter, randomized, double-blind, placebo-controlled (FLIGHT-FXR) | 10, 30, 60, 90 (Part A, B) 140 or 200 μg/day (Part C) PO | 2016-Completed   |
| Cilofexor        | FXR agonist         | 140 with NASH and fibrosis stage 0-3                                     | Phase 2, randomized, double-blind, placebo-controlled                      | 30 or 100 mg/day PO    | 2016-Completed   |
| EDP-305          | FXR agonist         | 134 with NASH and fibrosis stage 1-3                                     | Phase 2, randomized, double-blind, placebo-controlled, dose-ranging         | 1 or 2.5 mg/day PO     | 2018-Completed   |
| VK2809-201       | THR-β agonist       | 59 with NAFLD and hypercholesterolemia                                   | Phase 2, multicenter, randomized, double-blind, placebo-controlled          | 5, 10 mg/day or 10 mg QOD PO | 2016-Completed   |

*Fc-FGF21, human IgG1 fusion protein-fibroblast growth factor 21; NASH, nonalcoholic steatohepatitis; PPARs, peroxisome proliferator-activated receptors; PO, Per Os; FXR, farnesoid X receptor; THR-β, thyroid hormone receptor β; GLP-1, glucagon-like peptide; SC, subcutaneously; QOD, quaque altera die; GIP, glucose-dependent insulinotropic peptide; ACC, acetyl-CoA-carboxylase*
Taking into account the complex pathophysiology of NASH and the limited efficacy of the agents tested thus far, it seems reasonable that a one-drug approach might not be appropriate. Combining drugs that have complementary mechanisms of action might increase treatment efficacy. In this context, regimens that include a drug with a primarily metabolic mechanism of action (for example by targeting PPARs), combined with a drug that has anti-inflammatory or antifibrotic mechanisms of action (for example by targeting FXR), might help increase or maximize response rates [71]. It is important to note that drugs that have been already evaluated as monotherapy without showing individual effects should not be discarded, as they may exert synergistic effects in a combination regimen. Another way that combination treatment might be helpful is by decreasing the side effects of a certain drug that may otherwise limit its overall performance.

Lastly, the inclusion of antidiabetic drugs, in particular those that lead to a significant weight loss, like the GLP-1 analogs, may help improve both liver-related and diabetes-related outcomes.

Despite the undoubted appeal of combining drugs for the treatment of NASH, there is a need for more research to determine the right combination of pharmacological agents. The choice of drugs for future combination trials remains open. Therefore, the results from the ongoing trials will have a significant impact on future trials. Regarding the chronological sequence of the drugs in a combination regimen, currently the most prominent is concurrent therapy, when the combination is given from the start to the end of the treatment. Nevertheless, alternative strategies might also be successful, either in an outlasting manner, when a drug is stopped after a period of time, leaving another as a maintenance treatment, or in an addition manner, when a second drug is prescribed when the first loses its efficacy.

The majority of the ongoing combination trials for the treatment of NASH include an FXR agonist (Table 4). In a “proof-of-concept” study, 20 patients with NASH received cilofexor 30 mg (an FXR agonist) plus firsocostat 20 mg (an acetyl-CoA carboxylase [ACC] inhibitor with mainly antisteatotic effects) q.d. for 12 weeks (NCT02781584). A significant percentage of patients (74%) had a >30% decrease in liver fat, as determined by MRI-PDFF, and serum ALT and γ-glutamyltransferase were significantly improved. Subsequently, the safety and efficacy of this combination were assessed in a phase 2, randomised, double-blind, placebo-controlled study (the ATLAS study), which evaluated dual-combination regimens of cilofexor 30 mg, firsocostat 20 mg and selonsertib 18 mg (an apoptosis signal-regulating kinase 1 [ASK1] inhibitor with anti-inflammatory and antifibrotic effects) in patients with advanced fibrosis and cirrhosis due to NASH [72]. In a total of 392 treated patients (56% had compensated cirrhosis), a ≥1-stage improvement

### Table 4 Combination therapy in phase 2 clinical trials for NAFLD and/or NASH

| Drug name                  | Mechanism of action | Subjects | Study title                                      | Dose                      | Enrolment period |
|----------------------------|---------------------|----------|-------------------------------------------------|---------------------------|------------------|
| PF-06865571 and PF-05221304| DGAT2 inhibitor and ACC inhibitor | 99 with NAFLD | Phase 2a, randomized, double-blind, placebo-controlled, parallel-group | 30 mg/day and 600 mg/day (Arm D) | 2020-Completed |
|                           |                     | 450 with NASH and fibrosis stage 2-3 | Phase 2, randomized, double-blind, double-dummy, placebo controlled, dose ranging, dose finding, parallel group (MIRNA) | 300 mg/day or 600 mg/day and 10 mg/day or 20 mg/day | 2020-Ongoing |
| Semaglutide, Firsocostat and Cilofexor | GLP-1 agonist, ACC inhibitor and FXR agonist | 109 with NASH and fibrosis stage 2-3 | Phase 2, randomized, open label, proof-of-concept | 2.4 mg/week SC, 20 mg/day and 30 mg/day or 100 mg/day PO | 2020-Completed |
|                           |                     | 440 with compensated cirrhosis due to NASH | Phase 2, randomized, double-blind, double-dummy, placebo-controlled | 2.4 mg/week SC, 30 mg/day and 20 mg/day PO | 2021-Ongoing |
| Tropifexor and Cenicriviroc | FXR agonist and CCR2/5 antagonist | 193 with NASH and fibrosis stage 2-3 | Phase 2, multicenter, randomized, double-blind, placebo-controlled, long-term (TANDEM) | 140 µg/day or 90 mg/day and 150 mg/day PO | 2020-Completed |

NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease; DGAT2, diacylglycerol O-acyltransferase 2; ACC, acetyl-CoA carboxylase; PO, per os; FXR, farnesoid X receptor; CCR2/5, chemokine C-C receptors 2 and 5; GLP-1, glucagon-like peptide; SC, subcutaneously
in fibrosis without worsening of NASH after 48 weeks of treatment was numerically higher in all combination groups. Furthermore, the combination of cilofexor plus firsocostat led to statistically significant decreases of ≥2 points in NAS score, serum ALT and serum-based noninvasive fibrosis markers, compared with placebo. Finally, the combination of cilofexor plus firsocostat along with the GLP-1 agonist semaglutation has been investigated in a phase 2 proof-of-concept, open-label study (NCT03987074). This combination showed significantly higher reductions in hepatic fat fraction than semaglutation alone [73].

Another FXR agonist, tropifexor, is also being investigated in several combination trials. The phase 2 TANDEM trial assesses the combination of cenicriviroc with 2 doses (90 or 140 μg) of tropifexor over 48 weeks in 200 patients with biopsy-proven NASH and fibrosis stage F2-F3 [74]. In another trial, tropifexor in combination with the SGLT1/2 inhibitor licogliflozin will be evaluated for 48 weeks as a treatment for adults with fibrotic NASH (NCT04065841).

As discussed earlier, the combination of different agents can ameliorate the side effects of a certain drug. In this context, 2 studies were designed with the aim of decreasing the negative effect that FXR agonists and ACC inhibitors have in the lipid profile of the patients. The CONTROL study, a randomized, placebo-controlled, double-blind phase 2 study, showed decreases in LDL cholesterol when atorvastatin was added to OCA [75]. In the second study, the initiation of fenofibrate prior to the start of firsocostat prevented the increase in TG and improved hepatic fat and liver biochemistry in patients with advanced fibrosis due to NASH [76].

**Concluding remarks**

NASH is likely to become the leading cause of cirrhosis and etiology for liver transplantation in the western world. Several classes of therapies have shown promise, and are currently being evaluated in large phase Ib and phase III trials. Combination therapy, in which 2 or more drugs target different mechanistic pathways, could also boost the clinical response. As NASH is a heterogeneous disease, targeting multiple mechanistic pathways could achieve an optimal treatment response. However, more research is needed to determine the choice of drugs for a potential combination trial. Histologic assessment remains the cornerstone of assessing treatment response. Long-term follow up is necessary to demonstrate clinical benefits in improving liver-related outcomes. Further advances in noninvasive assessment are needed to improve the efficiency of clinical drug development in NASH and its related cirrhosis.

**References**

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-

Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;64:73-84.

2. Younossi ZM, Marchesini G, Pinto-Cortez H, Petta S. Epidemiology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: implications for liver transplantation. *Transplantation* 2019;108:22-27.

3. Younossi ZM. The epidemiology of nonalcoholic steatohepatitis. *Clin Liver Dis (Hoboken)* 2018;11:92-94.

4. Afsahi A, Forouzanfar MH, Reitsma MB, et al; GBD 2015 Obesity Collaborators. Health effects of overweight and obesity in 195 countries over 25 years. *N Engl J Med* 2017;377:13-27.

5. Loomba R, Friedman SL, Shulman G. Mechanisms and disease consequences of nonalcoholic fatty liver disease. *Cells* 2021;10:2537-2564.

6. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018;67:328-357.

7. Sanyal AJ, Van Natta ML, Clark J, et al; NASH Clinical Research Network (CRN). Prospective study of outcomes in adults with nonalcoholic fatty liver disease. *N Engl J Med* 2021;385:1559-1569.

8. Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018;15:11-20.

9. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011;34:274-285.

10. Parthasarathy G, Revelo X, Malhi H. Pathogenesis of nonalcoholic steatohepatitis: an overview. *Hepatol Clin* 2020;4:478-492.

11. Yuppalanchi R, Nourreddin M, Alkhouri N, Sanyal AJ. Therapeutic pipeline in nonalcoholic steatohepatitis. *Nat Rev Gastroenterol Hepatol* 2021;18:373-392.

12. Nourreddin M, Sanyal AJ. Pathogenesis of NASH: the impact of multiple pathways. *Curr Hepatol Rep* 2018;17:350-360.

13. Johnston MP, Patel J, Byrne CD. Multi-drug approaches to NASH: what’s in the development pipeline? *Expert Opin Investig Drugs* 2020;29:143-150.

14. Mota M, Banini BA, Cazanave SC, Sanyal AJ. Molecular mechanisms of lipotoxicity and glucotoxicity in nonalcoholic fatty liver disease. *Metabolism* 2016;65:1049-1061.

15. Lim JS, Mietus-Snyder M, Valente A, Schwarz JM, Lustig RH. The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. *Nat Rev Gastroenterol Hepatol* 2010;7:251-264.

16. Koliaki C, Szendroedi J, Kaul K, et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. *Cell Metab* 2015;21:739-746.

17. Caligiuri A, Gentilini A, Marra F. Molecular pathogenesis of NASH. *Int J Mol Sci* 2016;17:1575.

18. Fromenty B, Robin MA, Igoudjil A, Mansouri A, Pessayre D. The ins and outs of mitochondrial dysfunction in NASH. *Diabetes Metab* 2004;30:121-138.

19. Schwarz DS, Blower MD. The endoplasmic reticulum: structure, function and response to cellular signaling. *Cell Mol Life Sci* 2016;73:79-94.

20. Szegedi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep* 2006;7:880-885.

21. Schuster S, Cabrera D, Arrese M, Feldstein AE. Triggering and resolution of inflammation in NASH. *Nat Rev Gastroenterol Hepatol* 2018;15:349-364.

22. Meli R, Mattace Raso G, Calignano A. Role of innate immune response in non-alcoholic fatty liver disease: metabolic complications and therapeutic tools. *Front Immunol* 2014;5:177.

23. Miura K, Yang L, van Rooijen N, Brenner DA, Ohsishi H, Seki E.
Toll-like receptor 2 and palmitic acid cooperatively contribute to the development of nonalcoholic steatohepatitis through inflammasome activation in mice. *Hepatology* 2013;57:577-589.

24. Rahman K, Desai C, Iyer SS, et al. Loss of junctional adhesion molecule a promotes severe steatohepatitis in mice on a diet high in saturated fat, fructose, and cholesterol. *Gastroenterology* 2016;151:733-746.

25. Okin D, Medzhitov R. The effect of sustained inflammation on hepatic mevalonate pathway results in hyperglycemia. *Cell* 2016;165:343-356.

26. Spruss A, Kanuri G, Wagnerberger S, Haub S, Bischoff SC, Bergheim I. Toll-like receptor 4 is involved in the development of fructose-induced hepatic steatosis in mice. *Hepatology* 2009;50:1094-1104.

27. Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. *Nat Rev Gastroenterol Hepatol* 2017;14:397-411.

28. Yu J, Marsh S, Hu J, Feng W, Wu C. The pathogenesis of nonalcoholic fatty liver disease: interplay between diet, gut microbiota, and genetic background. *Gastroenterol Res Pract* 2016;2016:2862173.

29. Musso G, Cassader M, Paschetta E, Gambino R. TM6SF2 may drive postprandial lipoprotein cholesterol toxicity away from the vessel walls to the liver in NAFLD. *J Hepatol* 2016;64:979-981.

30. Joshi-Barve S, Kirpich I, Cave MC, Marsano LS, McClain CJ. Alcoholic, nonalcoholic, and toxicant-associated steatohepatitis: mechanistic similarities and differences. *Cell Mol Gastroenterol Hepatol* 2015;1:356-367.

31. Bourrier J, Mueller O, Barret M, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 2016;63:764-775.

32. Farhadi A, Gundlapalli S, Shaikh M, et al. Susceptibility to gut leakiness: a possible mechanism for endotoxaemia in non-alcoholic steatohepatitis. *Liver Int* 2008;28:1026-1033.

33. Bashir S, Shapiro H, Rozin S, Shibolet O, Elinav E. Fatty acid-bile acid conjugate Aramchol reduces liver fat content dithiolethione in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2017:45:1038-1048.

34. Fuchs CD, Traussnigg SA, Trauner M. Nuclear receptor modulation for the treatment of nonalcoholic fatty liver disease. *Semin Liver Dis* 2016;36:69-86.

35. Younossi ZM, Loomba R, Rinella ME, et al. Current and future therapeutic regimens for non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). *Hepatology* 2018;68:361-371.

36. Kim W, Kim BG, Lee JS, et al. Randomised clinical trial: the efficacy and safety of olipraz, a liver X receptor alpha-inhibitory dithiolethione in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2017;45:1073-1083.

37. Safadi R, Konikoff FM, Mahamid M, et al; FLORA Group. The fatty acid-bile acid conjugate Aramchol reduces liver fat content in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2014;12:2085-2091.e1.

38. Konikoff FM, Gilat T. Effects of fatty acid bile acid conjugates (FABACs) on biliary lithogenesis: potential consequences for non-surgical treatment of gallstones. *Curr Drug Targets Immune Endocr Metabol Disord* 2005;5:171-175.

39. Leikin-Frenkel A, Gonen A, Shaish A, et al. Fatty acid bile conjugate inhibits hepatic stearyl coenzyme A desaturase and is non-atherogenic. *Arch Med Res* 2010;41:397-404.

40. Ratziu V, de Guevara L, Safadi R, et al; ARREST investigator study group. Aramchol in patients with nonalcoholic steatohepatitis: a randomized, double-blind, placebo-controlled phase 2b trial. *Nat Med* 2021;27:1825-1835.

41. Fougerat A, Montagner A, Loiseau N, Guillou H, Wähl H. Peroxisome proliferator-activated receptors and their novel ligands as candidates for the treatment of non-alcoholic fatty liver disease. *Cells* 2020;9:1638.

42. Choudhary NS, Kumar N, Duseja A. Peroxisome proliferator-activated receptors and their agonists in nonalcoholic fatty liver disease. *J Clin Exp Hepatol* 2019;9:731-739.

43. Sven M F, Pierre B, Manal F A, et al. A randomised, double-blind, placebo-controlled, multi-centre, dose-range, proof-of-concept, 24-week treatment study of lanifibranor in adult subjects with non-alcoholic steatohepatitis: Design of the NATIVE study. *Contemp Clin Trials* 2020;98:101670.

44. Francque SM, Bedossa P, Ratziu V, et al. A randomized, controlled trial of the pan-PPAR agonist lanifibranor in NASH. *N Engl J Med* 2021;385:1547-1558.

45. Adorini L, Pruzanski M, Shapofo D, Farnesoid X receptor targeting to treat nonalcoholic steatohepatitis. *Drug Discov Today* 2012;17:988-997.

46. Neushanser-Tetri BA, Loomba R, Sanyal AJ, et al; NASH Clinical Research Network. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015;385:956-965.

47. Younossi ZM, Ratziu V, Loomba R, et al; REGENERATE Study Investigators. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet* 2019;394:2184-2196.

48. Intercept Pharmaceuticals, Inc. Press release: Intercept receives complete response letter from FDA for obeticholic acid for the treatment of fibrosis due to NASH. 2020. Available from: https://ir.interceptpharma.com/news-releases/news-release-details/intercept-receives-complete-response-letter-fda-obeticholic-acid [Accessed 15 February 2022].

49. Sinha RA, Bruinstraoop E, Singh BK, Yen PM. Nonalcoholic fatty liver disease and hypercholesterolemia: roles of thyroid hormones, metabolites, and agonists. *Thyroid* 2019;29:1173-1191.

50. Harrison SA, Bashir MR, Guy CD, et al. Retemirogl (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2019;394:2012-2024.

51. Lau J, Bloch P, Schäffer L, et al. Discovery of the once-weekly glucagon-like peptide-1 (GLP-1) analogue semaglutide. *J Med Chem* 2015;58:7730-7738.

52. Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. *Cell Metab* 2013;17:819-837.

53. Flint A, Andersen G, Hockings P, et al. Randomised clinical trial: semaglutide versus placebo reduced liver steatosis but not liver stiffness in subjects with non-alcoholic fatty liver disease assessed by magnetic resonance imaging. *Aliment Pharmacol Ther* 2021;54:1150-1161.

54. Marso SP, Bain SC, Consoli A, et al; SUSTAIN-6 Investigators. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2016;375:1834-1844.

55. Newsome P, Francque S, Harrison S, et al. Effect of semaglutide on liver enzymes and markers of inflammation in subjects with type 2 diabetes and/or obesity. *Aliment Pharmacol Ther* 2019;50:193-203.

56. Newsome PN, Buchholtz K, Cusi K, et al; NN9931-4296 Investigators. A placebo-controlled trial of subcutaneous semaglutide in nonalcoholic steatohepatitis. *N Engl J Med* 2021;384:1113-1124.

57. Ferrannini E. Sodium-glucose co-transporters and their inhibition: clinical physiology. *Cell Metab* 2017;26:27-38.

58. Kuchay MS, Krishan S, Mishra SK, et al. Effect of empagliflozin on liver enzymes in patients with type 2 diabetes and nonalcoholic fatty liver disease: a randomized controlled trial (E-LIFT trial). *Diabetes Care* 2018;41:1801-1808.

59. Shimizu M, Suzuki K, Kato K, et al. Evaluation of the effects of
dapagliflozin, a sodium-glucose co-transporter-2 inhibitor, on hepatic steatosis and fibrosis using transient elastography in patients with type 2 diabetes and non-alcoholic fatty liver disease. *Diabetes Obes Metab* 2019;21:285-292.

61. Mantovani A, Petracca G, Csermely A, Beatrice G, Targher G. Sodium-glucose cotransporter-2 inhibitors for treatment of nonalcoholic fatty liver disease: a meta-analysis of randomized controlled trials. *Metabolites* 2020;11:22.

62. Gawrieh S, Loo NM, Awasty VR, et al. LO10: A phase 2, prospective, multicenter, double-blind, randomized study of saroglitazar magnesium 1 mg, 2 mg or 4 mg versus placebo in patients with nonalcoholic fatty liver disease and/or nonalcoholic steatohepatitis (EVIDENCES IV). In: AASLD Late-breaking Abstracts Proceedings, 2019.

63. Maratos-Flier E. Fatty liver and FGF21 physiology. *Exp Cell Res* 2017;360:2-5.

64. Harrison SA, Neff G, Guy CD, et al. Efficacy and safety of aldafermin, an engineered FGF19 analog, in a randomized, double-blind, placebo-controlled trial of patients with nonalcoholic steatohepatitis. *Gastroenterology* 2021;160:219-231.

65. Harrison SA, Neff G, Guy CD, et al. Final analysis of a 24-week, randomized, double-blind, placebo-controlled, multicenter study of aldafermin (NGM282) in patients with nonalcoholic steatohepatitis. *Hepatology* 2020;72(Suppl):55A.

66. Sanjol A, Charles ED, Neuschwander-Tetri BA, et al. Pegbelfermin (BMS-986036), a PEGylated fibroblast growth factor 21 analogue, in patients with non-alcoholic steatohepatitis: a randomised, double-blind, placebo-controlled, phase 2a trial. *Lancet* 2019;392:2705-2717.

67. Harrison SA, Ruane PJ, Freilich BL, et al. Efuxifermin in non-alcoholic steatohepatitis: a randomized, double-blind, placebo-controlled, phase 2a trial. *Nat Med* 2021;27:1262-1271.

68. Téllez-Sanz R, García-Fuentes L, Vargas-Berenguel A. Human galectin-3 selective and high affinity inhibitors. Present state and future perspectives. *Curr Med Chem* 2013;20:2979-2990.

69. Chalasani N, Abdelmalek MF, Garcia-Tsao G, et al. Belapectin (GR-MD-02) Study Investigators. Effects of belapectin, an inhibitor of galectin-3, in patients with nonalcoholic steatohepatitis with cirrhosis and portal hypertension. *Gastroenterology* 2020;158:1334-1345.

70. Loomba R, Neutel J, Mohseni R, et al. LBP-20-VK2809, a novel liver-directed thyroid receptor beta agonist, significantly reduces liver fat with both low and high doses in patients with non-alcoholic fatty liver disease: a phase 2 randomized, placebo-controlled trial. *J Hepatol* 2019;70(Suppl):E150-E151.

71. Dufour JF, Caussy C, Loomba R. Combination therapy for non-alcoholic steatohepatitis: rationale, opportunities and challenges. *Gut* 2020;69:1877-1884.

72. Loomba R, Nourreddin M, Kowdley KV, et al. ATLAS Investigators. Combination therapies including cirexfer and firsocostat for bridging fibrosis and cirrhosis attributable to NASH. *Hepatology* 2021;73:625-643.

73. Alkhouri N, Herring Jr. RW, Kabler H, et al. Safety and efficacy of combination therapies including semaglutide, cirexfer and firsocostat in patients with NASH. *Hepatology* 2020;Abstract LO2. Available from: https://aasl.confex.com/aasl/2020/meetingapp.cgi/Paper/24605 [Accessed 15 February 2022].

74. Pedrosa M, Seyedkazemi S, Franchue S, et al. A randomized, double-blind, multicenter, phase 2b study to evaluate the safety and efficacy of a combination of trofifexor and canicriviroc in patients with nonalcoholic steatohepatitis and liver fibrosis: Study design of the TANDEM trial. *Contemp Clin Trials* 2020;88:105889.

75. Pockros PJ, Fuchs M, Freilich B, et al. CONTROL: A randomized phase 2 study of obeticholic acid and atorvastatin on lipoproteins in nonalcoholic steatohepatitis patients. *Liver Int* 2019;39:2082-2093.

76. Lawitz EJ, Neff G, Ruane PJ, et al. Fenofibrate mitigates increases in serum triglycerides due to ACC inhibitor firsocostat in patients with advanced fibrosis due to NASH: a phase 2 randomized trial. *Hepatology (Baltimore, Md.)* 2019;70:1489A-1490A.