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Therapies for non-alcoholic fatty liver disease: A 2022 update

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Abstract

The incidence of non-alcoholic fatty liver disease (NAFLD) is rapidly increasing and lifestyle interventions to treat this disease by addressing the underlying metabolic syndrome are often limited. Many pharmacological interventions are being studied to slow or even reverse NAFLD progression. This review for hepatologists aims to provide an updated understanding of the pathogenesis of NAFLD, current recommended therapies, and the most promising treatment options that are currently under development.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is an epidemic affecting 20-30% of the global population, paralleling the rise of type 2 diabetes (T2DM) and obesity (1–4). Unfortunately, about one in five patients with NAFLD progress to non-alcoholic steatohepatitis (NASH). Of those patients with NASH, 10% develop cirrhosis. NAFLD is now the second leading cause of liver transplantation in the US (5,6). In patients diagnosed with NASH, cardiovascular events are the leading cause of morbidity and mortality. These patients are also at higher risk of developing hepatocellular, pancreatic, and colorectal carcinoma (6). The rapid rise in disease burden, increased utilization of healthcare resources, morbidity, and mortality mandates early and effective therapies for NAFLD.

The past decade has seen a variety of new medications targeting novel physiological pathways undergoing evaluation. They purport to halt, and in some cases, even reverse the fibrosis seen in NAFLD. In this review we provide the present pathophysiological understanding and therapeutic options for NAFLD, with a preview of medications on the horizon.

UNDERSTANDING THE SPECTRUM OF NON-ALCOHOLIC FATTY LIVER DISEASE

Definitions

NAFLD is a clinical diagnosis that requires the presence of lipids in ≥5% hepatocytes as seen on liver imaging or biopsy, without secondary causes of hepatic fat accumulation such as alcohol use. It consists of the clinical spectrum of disease, which ranges in severity from simple steatosis to cirrhosis. Simple steatosis, or non-alcohol fatty liver (NAFL), is defined as the presence of fat without hepatocellular injury or inflammation, and while it was initially defined as a benign disease, recent evidence suggests that almost 25% of these patients can develop fibrosis (7,8). As there is an increase in disease activity, NAFL can progress to NASH, which is defined as evidence of hepatocellular injury through detection of lobular inflammation and hepatocellular ballooning, with varying degrees of fibrosis(9). Recent consensus statements argue that NAFLD is more accurately described as MAFLD, or "metabolic associated fatty liver disease" and is the interplay of genetic, environmental, and metabolic factors that manifest in multiple ways(10), requiring a definition of inclusion rather than exclusion.

Patients with NAFLD including fibrosis are at the highest risk of adverse outcomes (e.g. progress to cirrhosis and hepatic decompensation) (11,12). The presence and extent of fibrosis is the strongest predictor of many liver-related outcomes such as liver-related death and overall mortality(13).

The need for a noninvasive clinical marker that can measure disease progression and prognosis is still present. The current gold standard to diagnose NAFLD is liver biopsy, which is invasive and can result in complications. Scoring systems to measure

disease activity include the FIB-4 score have high negative predictive value, but have overall moderate accuracy (14).

2. Measuring Disease Activity

The scoring systems used for patients with NAFLD are the NAFLD Activity Score (NAS), developed by the NASH Clinical Research Network (CRN), and the Steatosis Activity Score (SAF), proposed by the European-based Fatty Liver Inhibition of Progression (FLIP) consortium(9,15).

Both NAS and SAF look at hepatocyte ballooning, lobular inflammation, and steatosis. However, the NAS reports disease activity as a composite score, with breakdown as shown in Table 1. In the cases where NAS is used, fibrosis stage is then reported separately. Alternatively, the SAF includes fibrosis as part of its score.

NAFLD Activity Score (NAS)

Steatosis Activity Score (SAF)

Component

Scoring Range

Component

Scoring Range

Steatosis

0-3

Steatosis

0-3

Lobular Inflammation

0-3

Activity (lobular inflammation + ballooning)

0-8

Hepatocyte ballooning

0-3

Fibrosis (separate from NAS)

Fibrosis (uses the same fibrosis staging as NAS)

3

F0

None

F1

Perisinusoidal or periportal

F1A

Mild, zone 3, perisinusoidal

F1B

Moderate, zone 3, perisinusoidal

F1C

Portal/periportal

F2

Both perisinusoidal and portal/periportal

F3

Bridging fibrosis

F4

Cirrhosis

Table 1. Comparing NAS and SAF

It is important to note that while both scores are used to grade disease severity and quantify the efficacy of interventions in clinical trials, they do not replace the analysis of histological patterns and subsequent diagnosis of the disease by a pathologist (2,9,16). Furthermore, both scores have limitations in their ability to fully assess patient response to treatment, due to inter- and intra-observer variability and "sampling error," due to the regional variability of disease activity within the liver itself(15).

PATHOGENESIS OF NAFLD

Hepatic lipid homeostasis is maintained through a variety of pathways. The main source of lipid uptake for the liver is *via* triglyceride lipolysis in adipose tissue, which releases fatty acids into the blood that are then taken up by the liver *via* membrane proteins called fatty acid transporters(17). The liver also performs de novo lipogenesis (DNL), through acetyl-coenzyme A, and DNL is regulated by enzymes such as sterol regulatory element binding protein 1c (SREBP-1c), a nuclear transcription factor, and peroxisome proliferator-activated receptor α (PPARα).

In the liver, fatty acids undergo either beta oxidation in the mitochondria to produce ketone bodies, which are then exported to the rest of the body as fuel, or undergo fatty acid esterification with glycerol to form inert triglycerides, which is released as VLDL or stored in hepatocytes as lipid droplets(18),(19).

The complex pathophysiology of NAFLD is driven by multiple hits. The major drivers include increased insulin resistance and impaired lipid metabolism. Other factors such as hormonal influences, gut-liver interactions, and genetics also play a significant role.

1. Insulin Resistance

Insulin resistance plays a key role in the development of NAFLD, and is a nearly universal feature of the disease(2). In hepatocytes, insulin inhibits gluconeogenesis, activates de novo lipogenesis, and promotes glycogen storage to decrease plasma glucose levels. In adipocytes, insulin promotes fatty acid esterification and lipid droplet storage while also

Insulin resistance (IR) is a defective metabolic response of target cell (hepatocyte, adipocyte inhibiting lipolysis(17)., skeletal muscle) to insulin, and develops mainly due to acquired factors such as obesity(18). It manifests as the ineffective suppression of lipolysis in adipose tissues, decreased glucose uptake by skeletal muscle due to the disruption of the translocation of the GLUT4 receptor to the surface membrane, and disturbed insulin mediated suppression of hepatic gluconeogenesis (18) (20).

In NAFLD, hyperinsulinemia is combined with inappropriately increased levels of peripheral lipolysis and de novo lipogenesis, contributing to increased circulating levels of free fatty acids and hepatic lipid burden. Furthermore, hormones that increase insulin sensitivity such as glucagon-like peptide-1 (GLP-1) have been reported to be decreased in patients with NAFLD (21).

2. Lipid Metabolism

Impaired intestinal permeability leading to increased translocation of intestinal toxins like lipopolysaccharides and ethanol has been reported in NAFLD. This can result in activation of hepatic macrophages releasing hepatotoxic factors like TNF-alpha (22).

The farnesoid X receptor (FXR) is a nuclear receptor located in the liver and small intestine that plays a key role in glucose metabolism, triglyceride synthesis, and bile acid flow regulation(19)(23), (24). Within the liver, FXR regulates hepatic triglyceride

synthesis *via* inhibition of SREBP-1c, thus decreasing lipogenesis. It also promotes free fatty acid oxidation and represses gluconeogenesis(24). Within the gut, FXR and its ligand, fibroblast growth factor 19 (FGF19), regulate bile acid synthesis by repressing CYP7A1, the enzyme for the rate limiting step in converting cholesterol to bile acids. Via the inhibition of CYP7A1 and de novo bile acid synthesis in the liver, the FXR/FGF19 pathway is an important component of bile acid synthesis and overall lipid metabolism (23), (25).

3. Gut Microbiota

There is increasing evidence that gut microbiome alteration and dysfunction contributes to NAFLD, T2DM, and obesity (26). Patients with these comorbidities have increased proportions of ethanol producing, Gram-negative microbes such as *Proteobacteria* and *Escherichia coli*, resulting in increased ethanol levels (27,28). Both bacteria itself and the ethanol activate production of Toll-like receptors (TLRs) and tumor necrosis factor (TNF) in the liver, which may drive NAFLD progression.

Furthermore, altered microbiota may contribute to inflammasome dysfunction, which has been associated with insulin resistance and obesity (29). Inflammasomes are protein complexes which sense DAMPs or PAMPs and process pro-inflammatory cytokines such as IL-1B and IL-18 (30). Unbalanced activation of these cytokines are associated with hepatic steatosis through increased TLR entry into the portal system (29).

4. Epigenetics

Genetics also plays a role in NAFLD, as familial aggregation, twin studies, and genome-wide association studies (GWAS) provide strong evidence NAFLD is an inheritable condition(31). Several genetic polymorphisms have been associated with NAFLD risk and severity, most notably the single nucleotide polymorphism (SNP) I148M of the PNPLA3 gene. Other genetic loci such as neurocan (NCAN), PPP1R3B, and glucokinase regulator (GCKR) have also been associated with steatosis in various GWAS (32,33). More research is needed to determine the exact mechanism of how epigenetic modifications can influence NAFLD pathogenesis.

5. Hepatic Inflammation

Insulin resistance, impaired intestinal motility, and impaired bile acid regulation, with underlying genetic alterations, all lead to a disruption of hepatic lipid homeostasis. Increased free fatty acid delivery to the liver results in increased VLDL secretion and generation of lipotoxic species(34) and decreased lipid removal. This sustained metabolic dysregulation maintains the ongoing low-grade systemic inflammation seen in NAFLD patients(18). This lipotoxicity causes damage-associated molecular pattern (DAMP) release that activates Kupffer cells and hepatic stellate cells, two of the resident hepatocyte immune cells. This triggers an immune system cascade that results in hepatic inflammation(17), (35).

This hepatic inflammation characterizes NASH, which can eventually progress to liver fibrosis, cirrhosis, and hepatocellular carcinoma. However, the pathophysiology behind how some patients simply develop steatosis while others progress to fibrosis and cirrhosis remains to be determined.

CURRENT RECOMMENDED INTERVENTIONS FOR NAFLD

There are no current FDA-approved medications for NAFLD. Lifestyle modifications of 5-10% weight loss through hypocaloric diets, aerobic exercise, and resistance training have been strongly advocated for by the AGA, AASLD, and EASL guidelines (9,36). A weight loss of $\geq 5\%$ of TBW can decrease hepatic steatosis, and $\geq 10\%$ weight loss has been shown to stabilize or even reverse fibrosis from NAFLD (36). This weight loss has been advocated through methods such as a hypocaloric diet, intermittent fasting, and aerobic exercise.

The Mediterranean diet (MD) has been the most extensively studied for patients with NAFLD. It is made up of a diet high in vegetables, fruit, legumes, minimally processed whole grains, fish, and nuts, with avoidance of dairy and red meat. In a study of twelve non-diabetic patients with NAFLD, the MD was found to reduce hepatic steatosis as measured on localized magnetic resonance H-spectroscopy (37). Furthermore, in a prospective analysis of 1521 participants in the Framingham Heart

Study, increased incorporation of the MD was found to be associated with reduced liver fat accumulation and odds of fatty liver incidence (38).

UPDATE ON THERAPIES UNDER EVALUATION

The present therapies under evaluation for NAFLD target the various stages of disease, with some having the possibility of reversing underlying fibrosis. Below we categorize therapies based on their role in the pathophysiological process of NAFLD.

Therapies Targeting Insulin Resistance

Since insulin resistance is one of the main drivers of NAFLD, obesity, and T2DM, and these diseases often coexist in the same patient, there have been several clinical trials to assess the efficacy of antidiabetic therapies. Currently, semaglutide and dapagliflozin are under phase 3 trials.

1a. GLP-1 receptor agonists

One promising therapy are GLP-1 receptor agonists (GLP-1RA), which stimulate fatty acid oxidation and gluconeogenesis, cause weight loss in diabetic and non-diabetic patients (39), improve glycemic control in patients with diabetes, and are associated with decreased cardiovascular risk. Exenatide, semaglutide, and liraglutide have all been studied.

Exenatide was initially studied in 44 obese patients with T2DM, who were initially given 5 μg twice daily and increased to 10 μg twice daily if well-tolerated. Compared to the placebo group, exenatide was found to reduce hepatic triglyceride content on MRI (-23.8 ± 9.5% *vs* +12.5 ± 9.6%, $P = 0.007$), most likely due to its weight loss effect ($r = 0.47$, $P = 0.03$) (40). When GLP-1RA showed benefit in weight loss and HgbA1c, further studies were conducted to assess their effect in patients with NASH.

Liraglutide was then studied in the LEAN trial, where 9/23 (39%) of patients with NASH who received subcutaneous liraglutide 1.8mg daily were found to have histological resolution of their disease, compared to 2/22 (9%) who received placebo (RR 4.3, 95%CI 1.0-17.7, $P = 0.019$). Furthermore, only 2/23 (9%) who received liraglutide had fibrosis progression, *vs* 8/22 (36%) in the placebo (RR 0.2, 95%CI 0.1-1.0,

$P = 0.04$). However, gastrointestinal side effects affected up to 31-81% of patients who received liraglutide (41).

Semaglutide is a GLP-1RA with more metabolic effects than liraglutide. In a recent multicenter phase 2 study of 320 patients with NASH, where 230 had stage F2-F3 fibrosis, those who received semaglutide 0.4mg weekly had a significantly higher rate of NASH resolution with no worsening of liver fibrosis (59% in 0.4-mg group vs. 17% in placebo group, OR 6.87, p<0.001) compared with those who received placebo. The authors also noted deceases in inflammatory biomarkers and weight loss in the semaglutide 0.4mg group. The main side effect was gastrointestinal disorders, but only 7% of the total study population discontinued the medication due to these side effects.

In the patients with F2 or F3 fibrosis, the confirmatory secondary endpoint of improvement in liver fibrosis with no NASH worsening was not met. It is postulated that this study may not have been long enough of a duration to truly assess fibrosis improvement, and since the patients in this study already had moderate-severe fibrosis, their condition would be harder to reverse(39). Further studies should also be conducted to assess for other factors as to why there were non-responders to this therapy, as genetics could also play a role. Currently, ESSENCE, a phase 3 trial involving patients with NASH, is assessing efficacy of semaglutide for steatohepatitis resolution and/or fibrosis improvement. The estimated trial completion date is in 2028 (NCT04822181) (42).

1b. SGLT-2 inhibitors

SGLT-2 inhibitors act in the kidney to promote urinary glucose excretion, causing improved insulin resistance in patients with T2DM. Canagliflozin, dapagliflozin, and empagliflozin are all SGLT-2 inhibitors that are in widespread use among patients with T2DM, and empagliflozin is also used for patients with cardiovascular disease.

In 37 patients with NAFLD and T2DM, canagliflozin 100mg daily was found to decrease hepatic fat content on MRI-PDFF (-6.9% [-9.5; -4.2] *vs* -3.8% [-6.3; -1.3] in placebo, $P = 0.05$), which correlated with weight loss ($r = 0.69$, p<0.001). It also increased

hepatic insulin sensitivity (p<0.01) (43). Dapagliflozin 10mg daily has been shown to decrease hepatic fat content on transient elastography in patients with T2DM and NAFLD and decrease fibrosis in patients with significant liver fibrosis, defined as LSM values ≥ 8.0 kPa (14.7±5.7 to 11.0±7.3, P = 0.0158). It has not yet been shown to increase insulin sensitivity of any organ (44,45). This suggests that canagliflozin could be utilized in patients with steatosis, while dapagliflozin may be more beneficial for patients with more fibrosis. Currently, a phase 3 trial is underway to assess dapagliflozin in patients with NAFLD, with an estimated completion year in 2023 (NCT 05308160) (46).

Empagliflozin was also studied in the phase 2 E-LIFT trial, where 50 patients (power ≥90%) with NAFLD and HgbA1c <10% received either empagliflozin 10mg daily or placebo. It is interesting to note that 40% of the study were women, and all were of Indian origin. The trial found that liver fat was significantly reduced in the empagliflozin group compared with the control (4.0% difference, p <0.0001). (47). A subsequent phase 4 trial further confirmed that patients on empagliflozin had decreased hepatic fat on MRI compared to placebo (relative decrease -22% [-36, -7], P = 0.009), but this was in patients with well-controlled T2DM (HgbA1c 6.6 ±0.5%) (48). Further multicenter studies are needed to assess the effectiveness of empagliflozin on patients with less-controlled T2DM and NAFLD.

1c. Insulin sensitizers

MSDC-0602K is a second-generation thiazolidinedione (TZD) insulin sensitizer. It targets the mitochondrial pyruvate carrier (MPC) with minimal PPAR☐ agonist binding, minimizing side effects seen with the original TZDs (e.g. edema and decreased bone density). MSDC-0602K was assessed in a phase 2b trial on patients with NASH and F1-F3 fibrosis, with the primary endpoint of achieving ≥2-point reduction in NAS. While histological effects were not statistically significant, it did improve glucose metabolism and liver enzymes, and patients were able to tolerate the drug with minimal side effects (49). Mice treated with combination MSDC-0602K and liraglutide therapy were found to have improved liver histology along with glucose tolerance,

suggesting that this may be a suitable combination for patients with T2DM and NASH (50).

Therapies Targeting Lipid Metabolism

Impaired lipid metabolism with resultant lipotoxicity is a key driver of hepatic inflammation and subsequent fibrosis. Key therapies that are currently in phase 3 trials include obeticholic acid, aramchol, and resmetirom.

2a. Farnesoid X receptor agonist

Obeticholic acid (OCA) is a farnesoid X receptor agonist, and has also been shown to reduce steatosis, liver weight, hepatic inflammation, and fibrosis in animal models, suggesting anti-inflammatory and anti-fibrotic effects. As a result, it is a promising therapy pending additional investigation of its side effects and tolerability. Other FXR agonists under investigation include cilofexor, which has been shown to decrease hepatic fat *via* MRI-PDFF, and tropifexor(24).

The FLINT trial, a multicenter double-blind, placebo-controlled, 72-week phase 2 trial assessed the effect of OCA in 283 patients with NAFLD with an NAS of 4, with a score of 1 or more in each component, with 225 patients with definite NASH at study entry(51). Study participants received either OCA 25mg or placebo, with 220 patients included in the primary outcome analysis, with both groups receiving standardized recommendations on lifestyle modifications. It demonstrated a statistically significant decrease in NAFLD activity score between the obeticholic acid *vs* placebo group, with higher rates of improvement in all three categories of the score. It also found a higher rate of improvement in hepatic fibrosis in patients receiving OCA *vs* placebo(24). The success of FLINT(23) led to REGENERATE(52), an ongoing phase 3 multicenter, randomized, placebo-controlled study that evaluated patients with non-cirrhotic NASH. This was defined as patients with biopsy-proven steatohepatitis, and NAS ≥4, with at least one point in each category. Their fibrosis stage was rated as F2 or F3, or F1 with at least one comorbidity (BMI ≥30, type 2 diabetes, or ALT >1.5 ULN), indicating that they had advanced fibrosis. Study participants received placebo, 10mg OCA, or 25mg OCA.

The 18-month interim analysis of the REGENERATE trial evaluated liver histology at month 18 as a prognostic indicator for clinical outcomes in a sample size of 750 patient and had 98% power. The intention to treat group analyzed for the primary analysis also included patients with more advanced fibrosis (F2-F3), and the group who received 25mg OCA met the primary endpoint of achieving a statistically significant improvement of fibrosis (reduction of at least one stage) with no worsening of NASH compared to placebo (23% in OCA 25mg *vs* 12% in placebo, *P* = 0.0002) (52). This was the first positive phase 3 study in patients with NASH fibrosis.

The main concerning side effects include elevated LDL-C and decreased HDL-C levels in the OCA 25mg *vs* placebo group. The CONTROL trial was a phase 2, double blind, 16-week trial that then evaluated the effects of gradual up titration of atorvastatin on 84 patients with NASH receiving OCA 25mg, 10mg, or placebo, starting from week four of OCA therapy. It found that with doses of atorvastatin 10mg, patients receiving OCA had increased LDL levels that decreased to below baseline(53). There was no clinical benefit seen with doses higher than atorvastatin 10mg. However, it is important to note that HDL-C and apolipoprotein A levels in OCA 25mg group remained unchanged between initiation of atorvastatin therapy and the end of the trial. Furthermore, 26% of these patients had compensated cirrhosis, and larger study sizes are needed to evaluate this medication regimen with a clear delineation between those with NASH *vs* cirrhosis.

There were also higher rates of pruritus (51%, 28%, and 19% in OCA 25mg, OCA 10mg, and placebo respectively), causing treatment discontinuation in 9% of those receiving OCA 25mg (compared to <1% in the OCA 10mg and placebo group). Other studies currently evaluating OCA include REVERSE, a phase 3 trial that is currently underway to evaluate the effect of obeticholic acid on patients with compensated cirrhosis due to NASH, which is expected to be completed in 2022 (NCT03439254).

2b. PPAR agonists

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that have been shown to be central for fatty acid metabolism, with pleiotropic effects on

glucose metabolism and fibrogenesis. The three different isotypes include PPARα (expressed in tissue with a high rate of fatty acid oxidation), β/δ (expressed in hepatocytes, Kupffer cells, hepatic stellate cells, and skeletal muscle), and γ (expressed in adipose tissue)(54).

Within the liver specifically, PPARα plays a key role in lipid metabolism, as it acts on hepatocytes and stellate cells to aid in beta-oxidation, thus reducing triglyceride levels in the liver and ameliorating hepatic lipotoxicity. It has also been shown to increase HDL levels. PPARβ/δ has systemic anti-inflammatory activity, as it regulates the expression of genes involved in innate immunity. PPARγ modulates fibrosis, as it prevents hepatic stellate cell activation, which is a key step in fibrogenesis, along with regulating insulin sensitivity. PPAR agents that have been evaluated include pioglitazone, one of the original therapies that were evaluated in a clinical trial; elafibranor, of which its phase three trial was terminated; and lanifibranor, which is currently in a phase three trial.

Pioglitazone, a PPARα/γ agonist and TZD, was one of the first drugs studied as a potential NASH therapy. The PIVENS trial was a 96-week study that compared NASH resolution in patients who received pioglitazone, vitamin E, or placebo(55). The study found that vitamin E was superior to placebo, and there was no benefit of pioglitazone over placebo for steatohepatitis improvement. This study suggests some benefit with using vitamin E as an adjunctive medication. It is also rarely prescribed due to multiple side effects such as weight gain, cardiac decompensation in patients with pre-existing conditions, and fluid retention.

A more promising drug is lanifibranor, a pan-PPAR agonist that was evaluated in the NATIVE trial (NCT 03008070), a multicenter, double-blind, placebo controlled 24-week trial with 247 participants who had NASH, SAF score ≥ 3, demonstrating that patients had high disease activity, and SAF steatosis score ≥ 1. Its primary endpoint was NASH improvement without worsening in fibrosis, as defined by a decrease from baseline of at least 2 points in the SAF and a stable or decreased CRN-F score, in

patients through evaluation of biopsies at baseline and at the end of the 24 wk period (54).

Patients were exposed to placebo, lanifibranor 800mg/day, or lanifibranor 1200mg/day. Based on the initial results, by the end of week 24, 63.9% of those receiving 1200mg lanifibranor met the primary endpoint, compared to 32.1% in the placebo group (RR 1.82, P = 0.004). Common side effects included gastrointestinal complaints, headache, and dizziness (56). A phase three trial to evaluate lanifibranor (800 mg and 1200mg once daily) vs placebo in patients with NASH and F2/F3 fibrosis has already been initiated with a primary composite endpoint of patients experiencing both NASH resolution and fibrosis improvement after a 72-week period.

The RESOLVE-IT trial (NCT 02704403) was a phase three trial which evaluated the effect of elafibranor, a PPARα/δ agonist, on histological improvement and all-cause mortality and liver-related outcomes in patients with NASH and fibrosis did not meet primary or secondary endpoint on its interim analysis and was terminated (57).

2c. Fibroblast growth factor agonist

As discussed early, the FXR/FGF19 pathway is a key regulator of energy metabolism. FGF19 has been shown to improve insulin sensitivity and increase adiponectin concentration in healthy obese patients with type 2 diabetes.

Pegbelfermin is an FGF19 analogue with a prolonged half-life that can allow for weekly dosing, which could also improve patient adherence, but its subcutaneous administration may also serve as a detracting factor. In a phase 2 trial evaluating 75 patients treated with subcutaneous injections of pegbelfermin 10mg daily, 20mg weekly, vs placebo daily, there was a significant effect of pegbelfermin on decreasing hepatic fat fraction in both groups during the interim analysis, as seen on MRI-PDFF (-6.8% in the 10-mg and -5.2% in the 20-mg group, compared to -1.3% in the placebo group). The study also found significant increases in adiponectin after pegbelfermin treatment (P = 0.0030), decreased LDL, and increased HDL(58). The study was terminated early due to these greater than expected results, so larger studies with

longer therapy duration are needed to assess efficacy in possible fibrosis improvement and monitor safety profile and side effects.

NGM282 is another FGF19 analogue that was studied in a randomized, double blind, placebo-controlled, 12-week long trial (NCT02443116) in 166 patients with NASH and an NAS≥4, stage 1-3 fibrosis, and at least 8% fat content. The primary endpoint was absolute change from baseline to week 12 in liver fat content, with a responder being categorized as someone with ≥5% absolute liver fat content reduction as seen on MRI-PDFF. NGM282 were associated with significant reductions in liver fat content (74% in the 3mg group, 79% in the 6mg group, *vs* 7% in the placebo, p<0.0001 for both comparisons). While these results warrant further study, it is also important to note the subcutaneous need for these injections, as the most commonly (≥10%) reported adverse events were injection site reactions (34%), along with diarrhea (33%), abdominal pain (18%), and nausea (17%)(59).

Efruxifermin, another long-acting FGF21 fusion protein, was also studied in a phase 2a trial of patients with NASH and F1-F3 fibrosis to assess its efficacy in hepatic fat reduction on MRI-PDFF. Patients in all treatment groups had a statistically significant decrease in hepatic fat content compared to placebo (-12% to -14%, p<0.0001) (60). 78% of patients also had ≥2 point reduction in NAS without worsening fibrosis, which is comparable to aldafermin, and this drug was generally tolerated.

2d. Stearoyl-CoA desaturase inhibitors

Stearoyl-CoA desaturase (SCD1) converts saturated fatty acids into monosaturated fatty acids and is key for hepatic lipogenesis. SCD1 downregulation has been shown to cause not just reduced hepatic lipogenesis, but also obesity resistance, enhanced insulin sensitivity, protection from steatosis, and enhanced lipid oxidation. In the 12 mo, global phase 2b randomized placebo-controlled ARREST trial, Aramchol, a stearoyl-CoA desaturase inhibitor(61), was studied in 247 patients with NAFLD (defined as NAS≥4), liver fat concentration of 5.5% or more as measured on MRS, and known T2DM (mean HgbA1c 6.6%) or pre-diabetes. Of the study population, 64.8% were women, and 63.2% were white. Patients received either Aramchol 400mg, 600mg,

or placebo, and the primary endpoint evaluated absolute reduction in liver fat *via* mean absolute change from baseline and ≥5% absolute reduction from baseline as seen on MR spectroscopy. While only patients on 400mg aramchol demonstrated a statistically significant mean absolute change from baseline in liver fat (400 *vs* placebo, P = 0.045; 600 *vs* placebo, P = 0.0655), patients on aramchol 600mg did demonstrate a ≥5% absolute reduction from baseline compared to placebo (47% *vs* 24.4%, P = 0.0279), and aramchol was found to be weight neutral without effects on lipid levels. The secondary endpoints of fibrosis improvement without worsening of NASH demonstrated an non-statistically significant improvement in those on aramchol 600mg *vs* placebo (29.5% *vs* 17.5%, P = 0.211), prompting the initiation of a phase 3 study, ARMOR (NCT 04104321), that is powered to evaluate NASH Resolution without worsening of liver fibrosis; or vice versa(62).

2e. Thyroid hormone receptor β agonist

Thyroid hormones also act in lipid metabolism. Thyroid hormone receptor (THR) α and β are distributed throughout the body, with β being the major one expressed in the liver. Thyroid hormone receptor beta is a key player in many of the pathways that regulate the pathogenesis of NASH. THRβ activation has been associated with reduction in triglycerides and cholesterol, improvement of insulin sensitivity, promotion of liver regeneration, and reduction of apoptosis. Resmetirom is a liver-selective, orally activated THR agonist, and is specifically uptaken by the liver. This is beneficial as its sole site of action will be on the liver, avoiding more systemic side effects of thyroid hormone receptor activation (62).

116 patients with biopsy proven NASH, NAS ≥4, fibrosis stage 1-3, and hepatic fat levels >10% as measured on screening MRI-PDFF were enrolled in a trial to study the effect of resmetirom on hepatic fat levels as measured with MRI-PDFF(62). However, it also important to note that up to 10% of patients could have either fibrosis stage 0, or hepatic fat levels at least 9% but less than 10%. All resmetirom patients were given 80mg doses for the first four weeks, and 24-h AUC exposures were calculated at week 2 and 4. At week 4 the AUC aided in titration of resmetirom dosing. The

treatment group was subdivided into a high exposure (resmetirom AUC≥2700 ng*h/mL) and low exposure (AUC<2700 ng*h/mL, but still associated with lipid lowering effects in phase 1 studies) subgroup. The study found that at week 12 and 36, resmetirom therapy was associated with significant reductions in relative and absolute hepatic fat fraction from baseline (-36.3%, p<0.0001). They also found that patients in the high exposure subgroup had greater relative hepatic fat reductions from baseline at week 12. Furthermore, the resmetirom group demonstrated reduced NASH features on liver biopsy, with a greater proportion of patients with ≥2 point reduction in NAS in the resmetirom group compared to placebo (46% *vs* 19% respectively, *P* = 0.017), and reduction in LDL, apolipoprotein B, and triglycerides. This suggests that along with improvement in hepatic fat, resmetirom may also decrease cardiovascular risk factors and improve histological features of NAFLD with minimal side effects of diarrhea and nausea that were mainly associated with therapy initiation. The phase three trial, MAESTRO-NASH, is currently underway in studying the effect of resmetirom on patients with NASH and F2-F3 fibrosis (NCT03900429) (63).

COMBINATION THERAPIES
Due to the complex pathophysiology of NAFLD, it is unlikely that there will be a single therapy for this disease.

A phase 2 of 108 patients with NASH evaluated safety of semaglutide 2.4 wkly only, *vs* in combination with cilofexor (30 or 100mg daily) and/or firsocostat 20mg daily. Patients had NASH based on biopsy with F2-F3 fibrosis or MRI-PDFF ≥10% and transient elastography (TE) liver stiffness ≥7 kPa (64). Although 73-90% of patients experienced adverse effects (mainly gastrointestinal), only 41-48% had ≥ grade 2 adverse events and only 8 (7.4%) discontinued their study drug. Exploratory endpoints found increased relative (-55.7 to -59.4% *vs* -46.2%) and absolute reductions (-9.8 to -11% *vs* -8%) in hepatic fat on MRI-PDFF in combination *vs* semaglutide groups. Based on liver stiffness assessment on TE, there was also potential reduction in hepatic fibrosis (mean change -2.29 to -3.74 kPa). A phase 2b trial with histologic endpoints is planned

to further assess safety and efficacy of these combination medications in patients with compensated NASH cirrhosis (NCT04971785) (65).

The TANDEM study was a phase 2b trial of 200 patients with biopsy-proven NASH and fibrosis F2-F3 to assess safety of tropifexor, an FXR agonist, and cenicriviroc, a chemokine receptor type 2/5 antagonist, compared to monotherapy (NCT03517540) (66). Results from this study have not yet been published.

Recently, an investigational combination therapy of ervogastat, a diacylglycerol acyltransferase 2 inhibitor, and clesacostat, an acetyl-coenzyme A carboxylase inhibitor, has been shown to be well-tolerated with a promising safety profile. It is currently being studied in a phase 2 trial of patients with biopsy-proven NASH with F2-F3 fibrosis (NCT04321031) (67).

CONCLUSION

Currently, a multitude of NAFLD therapies are in phase 3 trials including dapagliflozin, semaglutide, resmetirom, obeticholic acid, and aramchol, with more in development. The current trajectory likely involves tailoring drug therapies for different phases of NAFLD, such as utilizing aramchol or NGM282 for reduction of hepatic fat in patients with simple steatosis *vs* dapagliflozin in patients with fibrosis. Furthermore, combination therapies are also being studied in phase 2 trials. Due to the complex pathophysiology of NAFLD, these regimens will likely also be effective, but their safety, tolerability, and optimal drug combination must be assessed.

NAFLD is a disease with increasing prevalence and high rates of morbidity and mortality. Although lifestyle modifications remain an essential part of therapy, new and exciting drug regimens are on the horizon.

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