Future Treatment Options and Regimens for Nonalcoholic Fatty Liver Disease

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KEYWORDS

• Pharmacological treatment • NASH resolution • Fibrosis regression • Endpoints

KEY POINTS

- Assessing treatment efficacy in noncirrhotic nonalcoholic steatohepatitis currently relies on surrogate endpoints likely to predict clinically meaningful benefit.
- The failure of several drugs to hit their endpoints in phase 3 is in part reflective of the complex pathophysiology of the disease and the need to target the main drivers.
- Drugs that have a substantial impact on the metabolic drivers of the disease show promise but need further confirmation.
- Targeting several intrahepatic and extrahepatic key pathways simultaneously is probably required to achieve success in most patients.
- New compounds and innovative approaches are likely to change the therapeutic landscape in the near future.

INTRODUCTION

Although the progress in the field of nonalcoholic steatohepatitis (NASH) pharmacological treatment seems less spectacular compared with some other liver diseases, it has been significant and probably even decisive for future developments. A myriad of pathogenic studies aimed at identifying druggable targets (**Fig. 1**). The availability of candidate pharmacological agents and the flurry of NASH trials have provided the impetus for drug regulatory agencies to define a regulatory framework for drug

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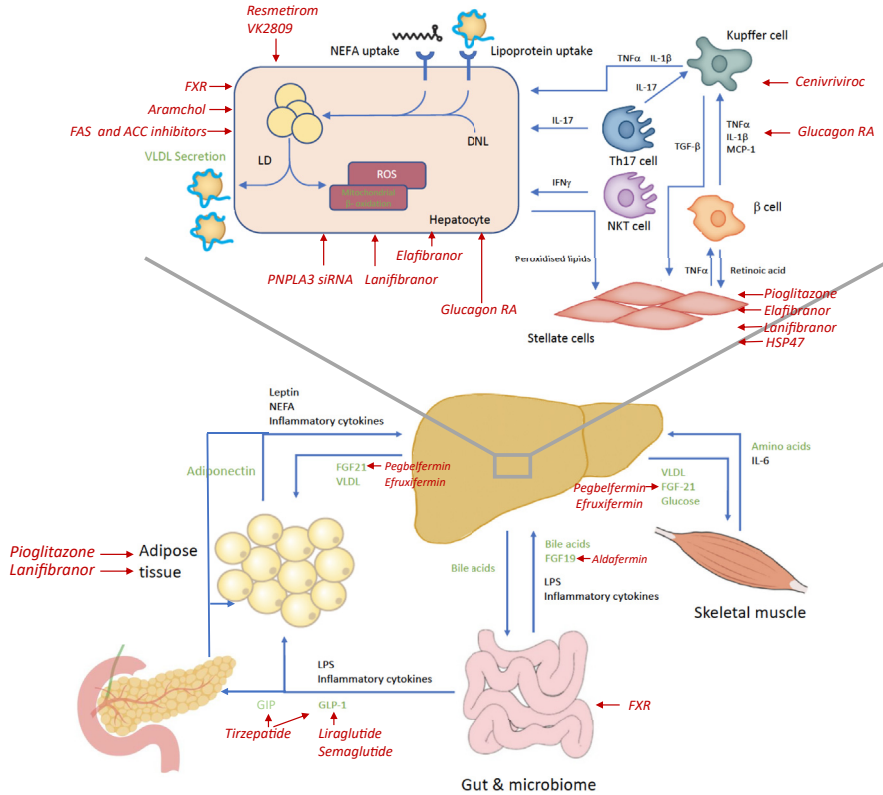

Fig. 1. Therapeutic targets in the complex pathophysiology of NASH. NASH is the result of a complex interplay of metabolic, inflammatory, and fibrogenic processes. Within the liver, hepatocytes and several of its intracellular organelles, most notably mitochondria, play an important role, alongside the stellate cells and several resident and infiltrating immune cells of different populations. NASH furthermore results from and affects an important crosstalk between the liver, the adipose tissue, the gut (including the gut microbiome), the muscle, and the pancreas. The cardiovascular system is also involved (not depicted, see[120]). Drugs that have been tested in NASH or that are under development have differential targets whether hepatic or extrahepatic. ACC, acetyl-CoA carboxylase; DNL, de novo lipogenesis; FAS, fatty acid synthase; FGF19, fibroblast growth factor 1; FGF21, fibroblast growth factor 21; FXR, farnesoid receptor X; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; IFNγ, interferon gamma; IL1-β, interleukin 1 beta; IL-6, interleukin 6; IL-17, interleukin 17; LD, lipid droplets; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein 1; NEFA, nonesterified fatty acids; NKT cell, natural killer T cell; PNPLA3, patatin-like phospholipase domain-containing protein 3; RA, receptor agonist; ROS, reactive oxygen species; siRNA, small interfering RNA; Th17, T helper 17 cell; TGFβ, tumor growth factor beta; TNFα, tumor necrosis factor alpha; VLDL, very-low-density lipoproteins. (Figure adapted from[117] [courtesy J. Haas] and [121].)

approval in NASH.[1] The recognition of fibrotic NASH as a serious and life-threatening condition has justified an accelerated approval pathway[2]; this allows a drug to be given conditional approval, while awaiting the evidence of clinical benefit required for definitive approval. The rationale is to ensure faster patient access to potentially

useful drugs in an area of unmet clinical need. Surrogate endpoints for conditional approval have been clearly defined: regression of fibrosis or resolution of NASH. These histological changes are achievable within a 12- to 18-month timeframe[3,4] and are therefore feasible within a trial context. Whether meeting these surrogate endpoints will result in clinical benefit has been questioned because no prospective demonstration is available yet; however, their use is currently supported by regulatory agencies.[2] Numerous retrospective studies have shown that fibrosis stage is associated with liver-related mortality and liver-related events,[5] and fibrosis stage reversal can even benefit patients with cirrhosis.[6] An important observation is that steatohepatitis itself increases the risk of liver-related events more than steatosis alone, even in the absence of fibrosis.[7] Moreover, changes in steatohepatitis status[8] (and, more widely speaking, in activity grade[9]) are positively associated with changes in fibrosis.[8,10] Thus, the chosen surrogates seem appropriate because they are achievable and have prognostic value. There are, however, caveats. Requiring complete NASH resolution could be unnecessarily strict, given the aforementioned relationship between changes in activity and changes in fibrosis. More importantly, documenting the disappearance of steatohepatitis, as defined by the absence of ballooned hepatocytes, can be very challenging, as even expert pathologists have difficulty agreeing on hepatocyte ballooning.[11] Finally, there are differences between European and American regulatory agencies regarding which combination of surrogate histological endpoints are acceptable.[12]

Clinical benefit required for definitive approval is typically tested in large long-term outcome trials[13] and is defined by mortality, liver transplantation, the occurrence of cirrhotic complications but also progression to compensated cirrhosis (defined clinically or histologically). In trials of midterm duration (typically 5 years) progression to cirrhosis is expected to be the most frequently occurring event. Although these outcomes have been repeatedly outlined by the regulatory agencies, the final decision relies not only on efficacy parameters but also on a complex assessment of the risk-benefit balance in the wider context of competing comorbidities in patients with NASH; this seems particularly relevant given that the chronic nature of the illness requires long-term therapy.

Recent Progress in Pharmacological Treatment

Over the past 15 years, many compounds have been explored for their utility in the treatment of NASH. A substantial number of trials have failed to meet prespecified primary endpoints. For some pathways, pharmacological intervention does seem to hold promise, and some new targets and approaches are currently explored.[14]

Farnesoid X Receptor Agonists

The farnesoid X receptor (FXR) plays an important role in bile acid metabolism but also on several metabolic, inflammatory, and fibrogenic pathways. FXR is present in the liver and the intestine, with some differences in effect according to the site.[15] Bile acids are the natural ligands of FXR. Ursodeoxycholic acid (UDCA) has no FXR agonistic effect, but the modified bile acid, obeticholic acid (OCA), is a potent FXR agonist currently licensed for the treatment of primary biliary cholangitis. In patients with NASH, the phase 2b Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment (FLINT) study[16] demonstrated improvement of histological activity of steatohepatitis as defined by a greater than or equal to 2 points reduction in NAS (a composite score of steatosis, hepatocyte ballooning, and lobular inflammation) but also a beneficial effect on fibrosis without, however, a significant effect on resolution of NASH. Importantly, OCA has been the first (and to date the only one) to subsequently

confirm the beneficial effects on fibrosis regression in the phase 3, REGENERATE trial (interim analysis on histological endpoints) after 18 months of treatment.[4] Nine hundred thirty-one patients with stage F2-F3 fibrosis were included in the primary analysis of this 3-arm trial. The fibrosis improvement endpoint was achieved by 12% of patients in the placebo group, 18% in the OCA 10 mg group (P = .045), and 23% in the OCA 25 mg group (P = .0002). These results were confirmed on a subsequent reading by different pathologists, including on several patients. NASH resolution is probably achievable given the effect of OCA on hepatocyte ballooning and lobular inflammation, although this endpoint was not formally met. Despite the efficacy on approvable surrogate endpoints, OCA is still not approved at the time of this writing, possibly because of a risk-benefit ratio that is still being assessed. OCA induces indeed several side effects such as dose-related pruritus, an increase in low-density lipoprotein (LDL) cholesterol (manageable with statin therapy) and biliary stones with cholecystitis. A black box warning has been issued due to several fatal cases mostly occurring following off-label prescriptions in patients with decompensated cirrhosis (Child-Pugh B or C) due to cholestatic diseases.

Several other bile acid FXR agonists are investigated, nor-UDCA being the most advanced (currently in phase 2) and promising. Non–bile acid FXR agonists are also actively developed with the prospect of reducing the unwanted side effects of OCA as a consequence of a lack of their enterohepatic cycle or due to different hepatic versus intestinal tropism or optimized pharmacokinetics. Compounds such as EDP-305[17] or MET-409[18] have confirmed a reduction in steatosis and biochemical efficacy but also pruritus as a dose-limiting, class effect, with, however, increases in LDL of a lesser magnitude.[19] Data from a 16-week trial of vonafexor, another nonsteroidal FXR agonist, suggested additional renal benefits to be further confirmed in larger trials.[20] Unfortunately, the only histological data available with a second-generation FXR agonist, a year-long trial of tropifexor, did not demonstrate efficacy when evaluated by traditional pathology, although assessment by digital pathology indicated patterns of fibrosis regression.[21] Tropifexor has been tested in a combination therapy with cenicriviroc and showed no benefit in favor of the combination,[22,23] although it is unclear whether this is due to the lack of efficacy of cenicriviroc.[24,25]

Fibroblast Growth Factors

As part of the FXR pathway, fibroblast growth factor (FGF) 19 is released by the intestinal cells on FXR stimulation, reaches the liver via the portal vein, and exerts its actions on bile acid metabolism via the FGF receptor 4 (FGFR4)/β-klotho (KLB) complex. FGFR4 is mainly expressed in the liver that confers a liver-targeted action of FGF19. FGF19 regulates hepatic bile acid synthesis by decreasing the expression of the rate-limiting enzyme (cholesterol 7 alpha-hydroxylase [CYP7A1]) of bile acid synthesis (which is also directly regulated by FXR).[26] FGF19 also affects lipid and glucose metabolism. However, by signaling via the IL-6/STAT3 pathway, FGF19 can drive tumorigenesis.[27]

NGM282 or aldafermin is an engineered FGF19 analogue that lacks the effect on the STAT3 pathway and hence most likely lacks the tumorigenic effect of FGF19. It demonstrated a significant reduction in liver fat content in a study including 82 patients with NASH.[28] A 24-week treatment in patients with F2-F3 fibrosis failed to reach the endpoint of fibrosis regression,[29]despite powerful suppression of toxic hydrophobic bile acids.[30] The drug is still under investigation in F4 patients (EudraCT Number: 2019–002341–38, NCT04210245).

FGF21 is another member of the FGF19 subfamily, also acting as a hormone. It is a so-called hepatokine, a peptide hormone mainly produced by the liver (but also by

multiple other organs, including the pancreas; circulating levels are, however, mainly determined by the hepatic production) regulating sugar intake, glucose homeostasis, and energy expenditure. It also needs the KLB coreceptor and acts mainly through the FGFR1c, which is mainly coexpressed with KLB in the central nervous system and adipocytes.[26] Interestingly, in view of the peroxisome proliferator–activated receptor (PPAR) drugs in the pipeline, its expression in the liver is regulated by PPARα. Animal data suggest enhanced NASH and associated metabolic derangements induced by FGF21 deficiency and improvement on FGF21 administration.[31] Interestingly, obese humans have increased circulating FGF21 levels, suggesting cellular FGF21 resistance.[32]

Recent data demonstrated a beneficial effect on liver fat content of Pegbelfermin, an injectable pegylated analogue of human FGF21, along with a reduction in biomarkers of liver injury and fibrosis. Phase 2b studies with histological endpoints at week 24 were, however, negative possibly due to a waning of the effect (tachyphylaxis) after the first few months of therapy.[33] Efruxifermin, a human FGF21 with 3 mutations fused to an immunoglobulin G1 Fc domain, showed strong antisteatogenic effects in an early study.[34] Recently released results from a 24-week study demonstrated significant improvements in NASH resolution and fibrosis regression over placebo.[35] A small uncontrolled study of pegozafermin, another pegylated FGF21, has shown NASH resolution or fibrosis reduction in 47% of patients after 20 weeks of therapy with a strong antisteatogenic and biochemical response including improvement of lipid parameters. These promising compounds are to be further tested in longer, phase 2b trials.

Peroxisome Proliferator–Activated Receptor Agonists

PPARs were first described as members of the steroid hormone receptor superfamily of ligand-activated transcription factors causing proliferation of peroxisomes. Peroxisomes play an important role in fatty acid catabolism and in the pentose pathway and hence in energy metabolism. They also play a role in the reduction of reactive oxygen species.[36] However, the actions of the PPAR target pathways involve several other cell organelles, most notably mitochondria. The pleiotropic actions of PPARs ultimately makes them critical regulators of not only fatty acid metabolism[37] but also glucose metabolism, inflammation, and fibrogenesis.[37,38]

Three PPAR isotypes have been identified (α, β/δ, and γ),[39,40] the expression and actions of which differ according to isotype, organ, and intraorgan cell type, resulting in a complex system of nuclear receptor–mediated interorgan crosstalk.[41] The main ligands for PPARs are fatty acids and their metabolites.

Despite preclinical rationale,[42] clinical data on PPARα single agonists are scarce. Fenofibrate reduces lipid levels by activating PPARα, which is highly expressed in the liver, but has no effect on insulin sensitivity[43] or hepatic steatosis.[44] Pemafibrate, which also showed benefits in preclinical nonalcoholic fatty liver disease (NAFLD) models and in patients with diabetes and dyslipidemia, also failed to reduce liver fat content.[45]

Thiazolidinediones (TZD) are PPARγ agonists that improve insulin resistance (IR) by direct effects on adipose tissue.[46] PPARγ activation in humans is associated with a broad spectrum of metabolic effects in great part derived from restoring adipose tissue biology[47,48] and a decrease in chronic systemic inflammation,[49,50] changes that are strongly associated with improvement in liver histology in patients with NASH.[51] In patients with prediabetes or type 2 diabetes (T2DM), pioglitazone 45 mg daily for 6 months improved NASH with a trend toward improvement in fibrosis compared with placebo.[52] A subsequent 18-month randomized controlled trial (RCT) in 101

patients with biopsy-proven NASH[53] confirmed these results. More recently, in an RCT of 105 patients with T2DM, pioglitazone plus vitamin E improved steatosis, hepatocyte ballooning, and inflammation.[54] The effect of pioglitazone on liver fibrosis is still unclear, as the landmark 2-year, PIVENS trial did not show an effect on fibrosis, instead all other histological parameters of steatohepatitis improved.[48]

The dual PPARα/γ agonist saroglitazar has beneficial effects in experimental models of NASH[55] and significantly decreases alanine transaminase levels and improves the cardiometabolic profile of subjects with biopsy-proven NASH.[56] A randomized, double-blind, phase 2 trial showed a significant effect on liver fat content after 16 weeks of treatment.[57] A study with histological endpoints is ongoing (NCT05011305).

The selective PPARβ/δ agonist seladelpar (MBX-8025) improves insulin sensitivity and steatohepatitis in mouse models of NAFLD[58] but its development in human NAFLD has been abandoned.

In rodent models of NASH and/or liver fibrosis, the dual PPARα/δ agonist elafibranor reduced liver fibrosis progression.[59] In the phase 2b GOLDEN 505 study of 274 noncirrhotic patients with biopsy-proven NASH, elafibranor 120 mg daily, was superior to placebo (20% vs 11%; $P = .018$) in patients with higher baseline NAFLD activity score (NAS $\geq$ 4).[60] Furthermore, a secondary post hoc analysis based on a revised definition for the resolution of NASH (with disappearance of ballooning and disappearance of lobular inflammation or persistence of mild lobular inflammation [score of 0 or 1], without worsening in liver fibrosis [progression by $\geq$ 1 stage]) was met with the 120 mg daily dose in the intention-to-treat population (19% vs 12%, $P = .045$).[60] Also, patients who improved NASH also improved fibrosis. However, the phase 3 RESOLVE-IT trial (NCT02704403)[61] did not confirm a significant benefit of elafibranor over placebo in inducing NASH resolution.[62]

The concept of combining PPARα, -β/δ, and -γ activation may represent a novel and potentially more efficacious therapeutic approach compared with single or dual agonists by targeting the large array of disturbances that contribute to the development and progression of NASH.[63,64] Lanifibranor (IVA337) is an indole sulfonamide PPAR agonist that activates all 3 subtypes α, β/δ, and γ, giving it the potential to address all the key features of NASH,[63] namely inflammation, steatosis, ballooning, and fibrosis.[65] In in vitro and in vivo preclinical studies, lanifibranor prevented and induced the regression of preexisting fibrotic damage in the liver and other organs without the classic effects on body weight, fluid retention, and heart weight increase reported with TZDs. Lanifibranor also improved metabolic features relevant to NASH.[63] A 24-week treatment with 800 or 1200 mg once daily showed dose-dependent significant effects on both resolution of NASH without worsening of fibrosis (45% for 1200 mg vs 19% on placebo, $P < .001$) and regression of fibrosis without worsening of NASH (42% vs 24%, $P = .011$) as well as on the composite endpoint of NASH resolution and fibrosis stage improvement (31% vs 7%, $P < .001$) with a good safety and tolerability profile.[66] The compound is now being tested in a large phase 3 study (EudraCT Number: 2020–004986–38, NCT04849728).

Incretins and Other Metabolic Hormones

Another approach is related to incretins and other hormones that are mainly known to handle body energy homeostasis and hence regulate glucose and lipid metabolism in the main involved organs. Glucagon-like peptide 1(GLP-1) is mainly secreted by intestinal L cells (mainly located in the ileum and colon) after exposure to nutrients. It stimulates insulin secretion by pancreatic β cells and inhibits glucagon secretion, hence contributing to the control of postprandial glycaemia. GLP-1 also affects on satiety

by action on the central nervous system and by slowing gastric emptying and intestinal transit. It also has a positive impact on beta-cell health and proliferation.[67]

Treatment with GLP-1 receptor agonist have shown considerable benefit in the treatment of diabetes and obesity and conferring long-term cardiovascular protection. Several studies have assessed their utility for the treatment of NASH. Data with histological endpoints come from a small 1-year trial with liraglutide and a larger 18-month trial with semaglutide.[3,68] Both studies show, besides improvements in body weight, glycemic control, and lipid profile, an improvement in liver histology in terms of features of steatohepatitis. Semaglutide at a daily dose of 0.4 mg subcutaneously resulted in a placebo-subtracted effect size of 42% for NASH resolution with no worsening of fibrosis, confirming previous observations with liraglutide.

Looking at the global picture of fibrosis improvers, stabilizers, or worseners, the overall picture suggests nevertheless a beneficial effect on fibrosis with numerically less fibrosis worseners and more improvers in semaglutide-treated versus placebo-treated patients, but without reaching the endpoint of 1-stage regression of fibrosis without worsening of NASH despite 18 months of treatment and a strong effect on steatohepatitis.[3]

An important question is hence to understand the drivers of improvement. As no GLP-1 receptors are present in the liver, it is likely that the observed results are attributable to the improvement of the metabolic milieu and hence indirect actions of the drug. Whether longer treatment will result in more pronounced effects on fibrosis remains to be demonstrated. Side effects are mainly gastrointestinal. The drug is currently in the phase 3 ESSENCE trial (EudraCT 2019–004594–44, NTC U1111–1244–3678).

Dual agonists are currently being tested associating an effect on glucagon, glucose-dependent insulinotropic polypeptide (GIP), or FGF21 receptors in addition to GLP1 receptors. Some of these drugs have shown particularly strong weight loss effects.[69] BI 456906 is a dual agonist of GLP-1 and glucagon (GCG) receptors. Of note, glucagon secretion as such is inhibited by GLP-1. The endocrine action of glucagon increases fatty acid disposal through beta-oxidation (and, possibly, energy expenditure) and can hence reduce body weight. The dual compound is hence expected to result in a more pronounced body weight compared with the individual molecules, which might also translate into a more pronounced improvement in liver histology. Furthermore, in contrast to GLP-1, glucagon receptors are present in the liver and GCG receptors might directly increase the fatty acid oxidation in the liver, potentially reducing the lipotoxicity. Glucagon also affects bile acid metabolism, inflammation, and immune cell activation.[70] Such partially direct intrahepatic effects might theoretically further enhance efficacy. The phase 2 trial is ongoing (EudraCT 2020–002723–11; NCT04771273).

Another neuroendocrine peptide hormone under study is amylin, which is cosecreted with insulin by pancreatic β cells in response to food intake and which affects several postprandial processes, for example, delays gastric emptying and suppresses of glucagon secretion.[71] Besides its glucoregulatory properties, amylin has binding sites in areas of the brain known to be involved in the regulation of energy homeostasis. Long-acting analogues are currently studied in combination therapies, more specifically with semaglutide (EudraCT 2020–003566–39; NTC NNC0174–0833).

Emerging data suggest that the gut-derived nutrient-induced incretin GIP operates at the interface of metabolism and inflammation. GIP is released after a meal by the K cells in the duodenum and ileum and, similar to GLP-1, increases pancreatic insulin secretion in a glucose-dependent manner. It also affects satiety and energy expenditure. GIP has, however, many other actions, mainly on different types of immune cells

and hence influences immune cell metabolism, with direct and indirect effects on adipose tissue depots and energy expenditure, as well as the liver.[72,73] Tirzepatide is a GLP1-GIP dual agonist currently studied in NASH with histological endpoints, after positive results on biomarkers in patients with T2D with markers indicative of the presence of NASH (EudraCT 2019–001550–26; NCT04166773).[74] A triple GLP-1/GIP/glucagon receptor agonist is even in development.[75]

De Novo Lipogenesis

De novo lipogenesis (DNL) is an important source of liver fat in the context of NAFLD. Aramchol, a fatty-acid/bile acid conjugate, is an inhibitor of hepatic stearoyl-CoA desaturase-1, a key enzyme in hepatic lipogenesis that converts saturated fatty acids into monounsaturated fatty acids. Aramchol has direct effects on both hepatocytes and hepatic stellate cells, in the latter associated with increased PPARγ expression and reduced expression of profibrotic genes.[76]

In a 52-week phase 2b study comparing 2 doses of Aramchol with placebo, besides numerically reducing liver fat as measured by MR spectroscopy, Aramchol, 600 mg, resulted in a numerically higher rate of NASH resolution without worsening of fibrosis compared with placebo (16.7 vs 5%, $P = .051$), an analysis restricted to patients with both baseline and end-of-treatment liver biopsy available.[77] Based on these results and the good safety and tolerability profile, the compound is now in phase 3 (EudraCT 2019–002073–56, NCT04104321).

Acetyl-CoA carboxylase (ACC) catalyzes the first step in DNL and modulates mitochondrial fatty acid oxidation. Increased hepatic DNL flux and reduced fatty acid oxidation are hypothesized to contribute to steatosis. Some proinflammatory cells also show increased dependency on DNL, suggesting that ACC may regulate aspects of the inflammatory response in NASH.[78] Firsocostat is a liver-targeted ACC inhibitor that was tested alone or in several combinations in patients with advanced fibrosis for 48 weeks. Albeit no statistical differences were observed compared with placebo, the combination of firsocostat with an FXR agonist had numerically a higher rate of fibrosis regression and also improved several features of steatohepatitis.[79] ACC inhibition typically results in hypertriglyceridemia, which can be mitigated by fibrates or DGAT2 inhibitors.[80] Several ACC1/2 inhibitors and combination therapies are under investigation.[81] Fatty acid synthase is a downstream target of lipogenesis that has been successfully modulated by a small molecule inhibitor, TVB-2640, with strong antisteatogenic and biochemical efficacy in an early phase NASH trial.[82]

Thyromimetics

Thyroid hormones increase energy expenditure and have catabolic properties, acting via the thyroid hormone receptor (THR), a nuclear receptor with different isoforms. In the liver, known effects of thyroid receptor activation on lipid metabolism include increased cholesterol metabolism via expression of the enzyme CYP7A1 and reduced DNL through suppressed expression of hepatic sterol regulatory element-binding protein-1.[83] An intrahepatic hypothyroidism is present in NASH and potentially contributes to its pathophysiology. This intrahepatocytic hypothyroidism is potentially attributable to alterations in hepatic deiodinase expression because of repair-related Hedgehog activation.[84] The incidence of clinical and subclinical hypothyroidism has also been reported to be higher in patients with NAFLD or NASH relative to age-matched controls.[85,86]

The THR agonist resmetirom (MGL-3196) has a selectivity for the TRH-β1 receptor (it is around 28 times more selective than triiodothyronine for THR-β vs THR-α) that is mainly expressed in the liver and the kidney, and therefore, resmetirom most likely

lacks some potentially important side effects of thyroid agonism, among others, on bone metabolism. Furthermore, this orally active compound is liver directed and is highly protein bound (>99%), with poor tissue penetration outside the liver.[87] In phase 2, resmetirom significantly reduced liver fat content after 12 weeks of treatment, with concomitant beneficial effects on liver enzymes and markers of liver inflammation, apoptosis, and fibrosis. Paired liver biopsies were obtained in 107 patients in the same trial after 36 weeks of treatment. NASH resolution without fibrosis worsening was significantly more frequent in the resmetirom arm compared with placebo, if patients with greater than 9.5% of weight loss (which only occurred in the placebo group) were excluded from the analysis, or when only MRI-PDFF responders were considered. No fibrosis improvement was detected histologically. There was no effect on body weight and glycemic control, but there was a significant improvement in atherogenic lipids (a decrease in triglycerides and LDL cholesterol, Lipoprotein (a), apolipoprotein [Apo]-B, and Apo-C3). There was no impact on bone mineral density. The compound is now further explored in several phase 3 trials (NCT04951219, NCT04197479, NCT03900429).[88]

Another compound with liver-specific thyromimetic properties is VK2809. VK2809 is a THR β-selective agent using a proprietary prodrug technology that confers lower systemic and tissue distribution relative to thyroid hormone and other thyromimetic drug classes. First-pass extraction of VK2809 in the liver, coupled with liver-specific activation to VK2809A and limited distribution of VK2809A to extrahepatic tissues, should confer a high degree of liver selectivity to the pharmacological effects of this agent. Currently, a phase 2 study with histological endpoints (as secondary endpoints) is ongoing (NCT04173065).[89]

Genetic Targets

Interfering with gene expression by small interfering RNAs (siRNA) is increasingly applied to treat disease. RNA interference is a naturally occurring cellular mechanism for regulation of gene expression: siRNA binds to its complementary messenger RNA (mRNA) sequence, which leads to mRNA cleavage and subsequent suppression of the synthesis and levels of the target protein.[90]

Hydroxysteroid 17β dehydrogenase 13 (HSD17B13) is a member of the 17β hydroxysteroid dehydrogenase (HSD) family, consisting of 14 structurally related enzymes implicated in steroid and fatty acid metabolism.[91] HDS17B13 is primarily expressed in the liver and is a lipid droplet enzyme with retinol dehydrogenase properties. It is highly expressed in patients with NAFLD compared with healthy controls, and variants of the *HSD17B13* gene have recently been associated with reduced hepatic injury, fibrosis, and inflammation among patients with NASH. The most common of these variants results in a truncated version of HSD17B13 protein that is catalytically defective and less stable, suggesting that the wild-type protein is associated with the development of NASH and its loss of function is protective against NASH and adverse outcomes among NASH patients.[92,93] ALN-HSD is an siRNA directly reducing hepatic *HSD17B13* expression, thereby mimicking the genetic loss of HSD17B13 function, which is expected to result in reduction of the hepatic injury in NASH. It is specifically designed for delivery into hepatocytes through conjugation of a carbohydrate ligand to the siRNA, resulting in uptake of siRNA via the asialoglycoprotein receptor after subcutaneous administration and is in early phase of development (NCT04565717). Results from an early trial have demonstrated a 90% inhibition of the expression of HSD17B13 mRNA.[94]

Another target of this approach is heat shock protein 47 (HSP47). HSP47 is a necessary component of the collagen deposition mechanism in myofibroblasts. By acting as

an intracellular chaperone of early collagen precursors for type I to type V collagens, HSP47 facilitates collagen formation, prevents collagen degradation, and ensures proper triple-helix formation of procollagen in the endoplasmic reticulum. Although HSP47 is not the initiating factor in the pathologic accumulation of extracellular collagen matrix, several experimental models have shown that HSP47 is required to produce high-quality collagen and that its intracellular levels increase with increased demand for collagen synthesis.[95,96] BMS-986263 is a lipid nanoparticle formulation that incorporates 6 lipid components and a retinoid-conjugated targeting agent (di-retinamide-PEG-di-retinamide [DPD]). The DPD moiety is present on the external surface of the nanoparticle and enables targeting and uptake by hepatic stellate cells via receptors for retinol-binding protein. The lipid nanoparticles contain a siRNA designed to suppress *HSP47* expression. The resultant decreased intracellular level of HSP47 should lead to a decrease in the formation of stable collagen and is hypothesized to result in decreased liver fibrosis. The compound has been tested in patients with hepatitis-C–related advanced fibrosis* and is currently studied in patients with NASH cirrhosis (NCT 04267393).[97]

The isoleucine-to-methionine substitution at position 148 (I148M) (rs738409C > G encoding for PNPLA3 I148M) in the patatin-like phospholipase domain-containing protein 3 (PNPLA3) is strongly associated with NAFLD and its severity and in particular also with the risk of hepatocellular carcinoma (HCC). Homozygosity for the common variant seems to confer a 10-fold increased risk of HCC in NAFLD.[98–100] The I148M PNPLA3 mutation has, however, also been shown to be of importance in other liver diseases.[101] The wild-type PNPLA3 protein has triglyceride and retinyl esters hydrolase activity. The 148M substitution induces a loss of function in this enzymatic activity, which results in an entrapment of triglycerides and retinyl esters in lipid droplets of hepatocytes and hepatic stellate cells.[102] The latter then induces liver damage. The mechanism is related to the accumulation of mutated 148M on lipid droplets, where it escapes proteasomal degradation and inhibits the activity of other lipases.[103] In mice fed an NASH-inducing diet, treatment with antisense oligonucleotide directed against PNPLA3 leads to improvements in steatosis. Furthermore, in mice carrying the human I148M mutant, antisense oligonucleotide treatment improves inflammation and fibrosis.[104] The aim is hence to reduce the overexpression of the I148M variant. Therefore, AZD2693, a compound with an siRNA lowering the mRNA expression of PNPLA3, is currently explored in patients who are homozygotes for the 148M risk allele.

Antifibrotic Drugs

As mentioned, fibrosis is an important target for therapy. Despite NASH being the driver of disease progression, fibrosis is the strongest predictor of liver-related outcomes as well as overall mortality.[105] Liver-related events also drive morbidity and mortality in patients with advanced fibrosis.[106] These patients do not decompensate and die from fibrosis *per se*, but fibrosis progression to cirrhosis results in the development of portal hypertension and hepatic insufficiency, which will drive cirrhosis decompensation. Fibrosis regression or even preventing fibrosis progression is therefore a rational objective, although to what extent this will translate in improved outcomes need to be studied.

Unfortunately, several drugs failed to demonstrate robust fibrosis regression, remarkably, all of them with a plausible antifibrotic mode of action. Cenicriviroc is a dual CCR2 and CCR5 antagonist. CCR2 and CCR5 play an important role in macrophage recruitment and polarization and have been implicated in NASH pathogenesis. Despite this rational mode of action, cenicriviroc did not show an effect on NASH

resolution,[17] and the significant benefit of cenicriviroc over placebo in terms of regression of fibrosis after 1 year of treatment[24] was unfortunately not confirmed with additional duration[25] or in a subsequent phase 3 trial. Simtuzumab is a lysyl oxidase homolog 2 antibody interfering with collagen cross-linking and is an example of a pure antifibrotic approach. Yet simtuzumab failed in 2 large NASH trials.[107] Selonsertib is an inhibitor of apoptosis signal-regulating kinase 1, which is involved in response to various cellular stresses. It was tested in a small, 6-month trial, alone or in combination with simtuzumab, and demonstrated a higher proportion of fibrosis regression versus the comparator[108] but without an effect on steatohepatitis, aminotransferases, or metabolic features. Unsurprisingly, the phase 3 trials failed to confirm a significant benefit in terms of fibrosis regression.[109]

WHAT EXPLAINS SO MANY FAILURES?

Several factors need to be considered to find an explanation for this high number of failures.[110] First, the trial endpoints being determined by histological reading, robust pathological assessment is crucial. Unfortunately, even the most experienced pathologists cannot eliminate the significant intra- and interobserver variability, let alone imprecisions or subjectivity in the definition or grading/staging of histological lesions.[11,111] However, a recent subanalysis of the semaglutide phase 2b trial analyzing histological changes by digital pathology yielded the same conclusions as the primary analysis. Although digital pathology does have a higher sensitivity to detect changes for different histological parameters, whether changes that are not detectable through conventional pathology are relevant for clinical outcomes remains to be determined. Another issue with the liver biopsy is sampling variability. Assessing a larger size biopsy or assessing several sections instead of a single slide can improve, although not completely mitigate, this issue but runs into serious feasibility issues in clinical trials. It should, however, be acknowledged that the noninvasive serum and imaging markers have been proposed as alternatives to the liver biopsy but these noninvasive diagnoses also have substantial variability.[112,113] Replacing an imperfect reference standard by another imperfect reference standard will not solve the issue. The limitations of the biopsy hence do not explain the whole picture.

Two other elements need to be considered. First, the definition of the endpoint only considers patients who show improvement in their fibrosis stage. However, halting disease progression might be as valuable, and absence of progression should also result in less progression to cirrhosis and hence liver-related events.[114] The placebo-subtracted effect size of OCA for fibrosis regression was 15% (in the per protocol analysis), whereas 8% less patients progressed compared with placebo, and thereby the overall antifibrotic benefit was higher than anticipated from fibrosis regression only.[4] Lanifibranor showed a comparable picture, with 14% and 5% placebo-subtracted effect size for regression and reduced progression of fibrosis, respectively.[66] The greater than or equal to 1-stage improvement in fibrosis is a high barrier endpoint. Despite numerical differences, semaglutide did not reach this endpoint, but here too fibrosis progression was slower on drug than on placebo, suggesting an overall beneficial impact on fibrosis.[115]

A second element brings back the concept of NASH as the driver of disease progression. Paired biopsy studies have shown that changes in fibrosis and disease activity occur in parallel.[9] One may therefore question if in order to obtain fibrosis regression it is not necessary to reduce disease activity. NASH resolution was the strongest predictor of fibrosis regression in a recent post-hoc analysis of NASH CRN trials,[8] and a subanalysis of the elafibranor phase 2 GOLDEN trial showed

correlation between changes in disease activity and fibrosis.[116] Lanifibranor not only induced fibrosis regression but also significantly induced NASH resolution, and OCA significantly improved ballooning and inflammation.[4,66] As mentioned, the strong effect of semaglutide on NASH resolution also was accompanied by a signal of beneficially affecting fibrosis evolution over time.[115] By contrast, cenicriviroc showed no effect on steatohepatitis[25] and had a questionable impact on fibrosis. Also, other compounds without an effect on steatohepatitis (eg, simtuzumab and selonsertib) failed to show an effect on fibrosis.[109] It is therefore likely that drugs that act only on mechanisms of fibrosis but that have no or insufficient effect on the upstream processes of metabolic dysfunction, cell damage, and inflammation may not achieve long-lasting fibrosis regression (**Fig. 2**).

Another legitimate question is whether compounds with a narrow mode of action tackling single pathways (eg, key enzymatic steps in de novo lipogenesis, blockage of a specific chemokine receptor subtype) might fail given the complexity of disease pathophysiology[117] and the existence of many other "rescue" or redundant pathways. Of course, combination therapies seem very appealing, despite having their own numerous challenges.

THE FUTURE OF NONALCOHOLIC STEATOHEPATITIS THERAPEUTICS

Given the complexity of its pathogenesis, there is great interest in the development of combination therapies targeting different aspects of NASH. Several elements will, however, need to be considered.

Antidiabetic drugs such as GLP-1 receptor agonists and sodium-glucose cotransporter-2 (SGLT2) inhibitors have proved efficacy in preventing cardiovascular or nephrological outcomes and are likely to be the background therapy for many diabetic patients with NASH in the near future. Although there are no data on hepatic histological benefit of SGLT2 inhibitors, GLP1 receptor agonists or dual or triple agonists are

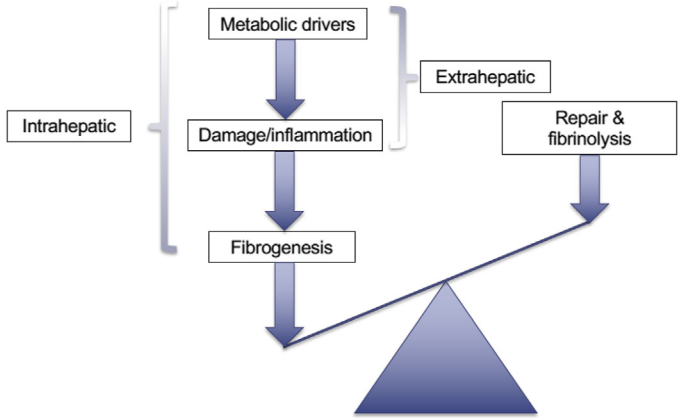

Fig. 2. Progression of disease in nonalcoholic steatohepatitis (NASH). Progression occurs when profibrogenic mechanisms are not sufficiently counteracted by repair mechanisms. Fibrosis is driven by processes of cell damage and inflammation, mostly initiated by lipotoxicity associated with adipose tissue dysfunction and insulin resistance. Thus, hepatic fibrosis is the end-stage adaptive response to injury. This makes the fibrogenic process difficult to control by a putative antifibrotic drug only, if upstream driving pathways are not being controlled. Ideally pleiotropic drugs or combinations thereof should act at several levels of this pathogenic sequence. (*Adapted from* Ref.[122])

actively tested for NASH. If the histological efficacy seen in early trials is confirmed, most diabetic NASH patients will be treated with these drugs, and compounds specifically developed for NASH and not for diabetes or weight loss will have to prove additional efficacy in order to be adopted. Combination strategies will have to consider specific approaches, such as induction and maintenance strategies versus intermittent cycled therapy versus sequential therapies depending on the potency and tolerability of future agents.

Also, given the biological complexity and clinical heterogeneity of NASH and its comorbidities, identification of the precise drivers of disease would support the development of targeted therapeutics. Understanding how precision or individualized medicine should be conducted will require studies on large numbers of well phenotyped and genotyped patients. The use of polygenic risk scores to identify individuals with specific risk characteristics and pathways of liver injury will be pivotal for successful implementation of personalized medicine approaches. Recent data from the Million Veterans Program[118] support this concept but currently do not go beyond providing insights on the development of fatty liver.[119] It will be crucial to integrate genomic, phenomic, and transcriptomic data to advance the field toward specifically targeted therapeutics that will benefit the largest number of patients with NASH.

DISCLOSURE

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