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Nonalcoholic Steatohepatitis (NASH) and Hepatic Fibrosis: Emerging Therapies

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Abstract

Nonalcoholic fatty liver disease remains a major cause of liver-related morbidity and mortality worldwide. It is a complex disease associated with obesity, diabetes, and dyslipidemia but is increasingly recognized in normal-weight individuals. Its progressive inflammatory phenotype, nonalcoholic steatohepatitis (NASH), currently has no effective treatment apart from lifestyle interventions. Multiple pathogenic pathways are involved in disease progression, and targets for intervention have been identified. These targets mediate glucose, lipid, and bile acid metabolism; inflammation; apoptosis; and fibrosis. Novel therapeutic agents are being developed in each of these pathways, and several have shown promise in early phase testing. Given the complexity of the disease, intervention trials are large and long and require histologic confirmation as a primary endpoint for disease improvement or regression. We highlight active Phase 2 and 3 therapeutic trials for NASH as this field rapidly expands in development.

INTRODUCTION

Nonalcoholic steatohepatitis (NASH) is emerging as the twenty-first century's imminent public health threat. Drug development in this area of chronic liver disease is explosive, given the large unmet need in addressing the underlying complex metabolic dysfunction associated with the disease and the clinical endpoint of cirrhosis (1). Unmitigated, cumulative fibrotic remodeling of the liver leads to cirrhosis, with consequences of increased liver-related mortality, development of cancer, or the need for liver transplantation. In the United States alone, prevalence estimates of nonalcoholic fatty liver disease (NAFLD) are approximately 20–30% in adults (2) and 10% in children (3, 4). The inflammatory phenotype, NASH, is present in approximately 3–5% of the Western population (5, 6), with notable differences according to method of diagnosis, age, sex, and ethnicity.

As a heterogeneous disease process that enters the health-care system via primary care, gastroenterology, endocrinology, cardiology, and hepatology clinics, the diagnosis and management of NASH may vary. Whereas the mainstay of treatment for the more benign NAFLD rests on weight loss through lifestyle intervention, the global burden of NASH provides the rationale for drug development and therapeutic trials, given the likelihood of liver-related outcomes (7). Current guidelines limit consideration of therapies targeting the liver to patients with biopsy-proven NASH, any fibrosis, or the existence of both histologic findings (8). These recommendations are based on natural history observations regarding the risk of progression to more advanced stages of liver disease (5, 9). The goal in halting or reversing NASH histologically is to limit disease progression.

A critical understanding of the known pathways involved in the development and progression of NASH undergirds the development of effective therapies. The efficacy of therapies must be considered in the context of clinically meaningful benefit in diverse populations. This is defined by how an affected patient feels, functions, or survives. Given the long duration needed for clinical outcomes to occur, short-term changes in histology have been accepted for drug approval but require long-term demonstration of a reduction in risk of progression to cirrhosis. The short-term histologic surrogates of such improvement include resolution of steatohepatitis, decrease in disease activity, and improvement in fibrosis stage (10). Tied to this improvement are the metabolic drivers of disease progression, which are more difficult to capture, as the metabolic profiles of patients who develop NASH vary quite markedly. The degree to which histology used as a surrogate marker of disease progression and regression will translate into hard outcomes with prevention of progression of liver-related disease and mortality will be determined only with longitudinal follow-up over many years. Given the slowly progressive nature of the disease, inclusion of individuals with more advanced stages of disease in whom hard outcomes can be predicted should be an area of priority.

POPULATIONS OF INTEREST

The current development of therapeutics in NASH has emphasized individuals with histologic changes characteristic of NASH, with a special emphasis on those with moderate to severe stages of fibrosis. Clinical outcomes associated with advanced fibrosis have focused efforts toward treating those with the greatest likelihood of progression toward cirrhosis. Prevalence of NAFLD varies among different ethnic groups, but progression and response to therapy in diverse populations merit additional exploration in clinical trials. Additional populations of interest for therapeutic intervention include those with NASH, in whom traditional recommendations for lifestyle intervention (e.g., weight loss, caloric restriction, exercise) might be less effective: those with normal weight (11), with limited caloric intake, and/or already engaged in regular physical activity as well as those for whom significant lifestyle changes cannot be maintained.

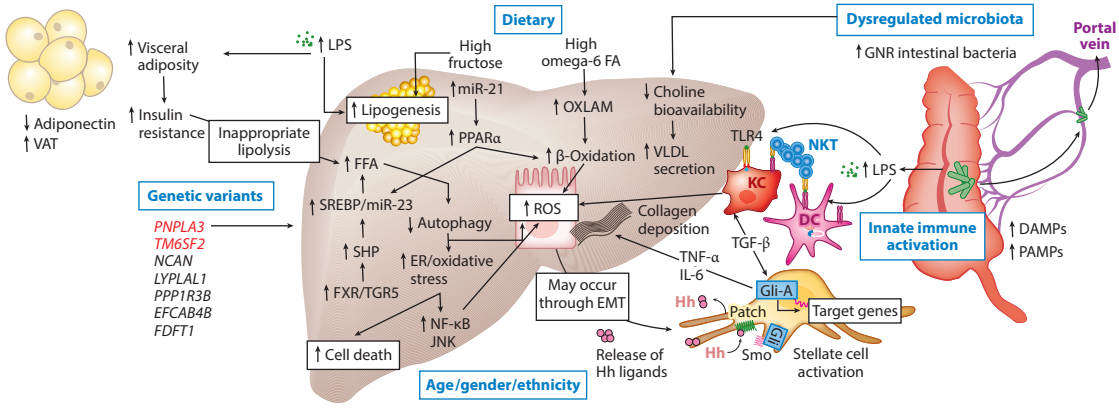

Figure 1

Pathogenesis of NAFLD/NASH. Abbreviations: DAMP, damage-associated molecular pattern; DC, dendritic cell; EMT epithelial mesenchymal transition; ER, endoplasmic reticulum; FA, fatty acid; FFA, free fatty acid; FXR, farnesoid X receptor; Gli-A, glioma transcription factor activator; GNR, gram-negative rod; Hh, Hedgehog; IL-6, interleukin 6; JNK, C-Jun N-terminal kinase; KC, Kupffer cell; LPS, lipopolysaccharide; miR, microRNA; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NF-κB, nuclear factor κB; NKT, natural killer T cell; OXLAM, oxidized linoleic acid metabolite; PAMP, pattern-associated molecular patterns; PPARα, peroxisome proliferator-activated receptor α; ROS, reactive oxygen species; SHP, small heterodimer partner; Smo, smoothened; SREBP, sterol regulatory element binding protein; TGR5, transmembrane G protein–coupled receptor 5; TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor-α; TNF-β, tumor necrosis factor-β; VAT, visceral adipose tissue; VLDL, very low density lipoprotein. Modified with permission from Stephen A. Harrison.

PATHOGENESIS OF NAFLD AND NASH

The underlying biologic mechanisms for this disease process are still not well known. A multiplicity of pathways are involved in the lipid accumulation, inflammation, cellular infiltration and fibrosis observed as the histologic hallmarks of the disease (see **Figure 1**). Current understanding of pathogenesis describes excess lipid substrate leading to lipotoxic liver injury. Metabolically overweight status, along with excessive fat accumulation in the visceral adipose tissue, causes insulin resistance. Consequently, inappropriate lipolysis occurs and shunts free fatty acids to the liver. Hyperinsulinemia further drives hepatic lipogenesis. Fatty acid oxidation is increased early but is impaired in late stages of the disease. The accumulation of lipids in the liver causes hepatocellular injury via several mechanisms including oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum (ER) stress. Cell stress requires the hepatocytes to adapt or undergo apoptosis. This stress triggers inflammatory signaling. Prolonged inflammation, coupled with cell death and regeneration, drives fibrogenic remodeling of the liver, culminating in cirrhosis (12, 13). A complex cross talk between adipose tissue, the liver, the gut (14), and the microbiome (15) superimposed on genetic risk factors (16) and environmental influences (nutritional intake and physical activity) (17) highlights several additional pathways for understanding underlying NAFLD biology in addition to potential therapeutic intervention. Key regulators of these pathways are now targets for therapeutics; active therapeutic trials that relate to these pathways are discussed in this review.

LIFESTYLE INTERVENTION

Although the focus of this review is pharmacologic intervention, it is important to stress that weight loss is the most effective intervention to improve NASH. Weight loss of >9% has shown

histologic improvement, but for the majority of patients with NASH, sustaining this weight loss over time has proved difficult (18). Recent studies have emphasized the importance of aerobic activity (19), but the role of aerobic and resistance activity in long-term disease modification has yet to be investigated in a large longitudinal cohort.

EMERGING THERAPIES CURRENTLY UNDER STUDY FOR THE TREATMENT OF NASH

Table 1 lists currently enrolling clinical trials in NASH available in **https://ClinicalTrials.gov** as of fall 2017. This review focuses on trials that are currently in progress.

NASH Therapies that Target Glucose and Fatty Acid Metabolism

Altered lipid metabolism (uptake and storage, biosynthesis, and excretion) within the liver is associated with NAFLD, as lipid accumulation within the liver is required for hepatic steatosis. Insulin resistance is well established to promote peripheral lipolysis, de novo lipogenesis, and thus hepatic steatosis—a necessary component of the disease and its development (20). Given the understanding that insulin resistance is central to the pathogenesis in NASH, drugs that act to improve insulin sensitivity are credible, evidence-based treatment candidates. The peroxisome proliferator-activated receptor γ (PPARγ) isotype presents predominantly in the adipose tissue. The thiazolidinedione class improves insulin resistance through effects in the liver, muscle, and adipose tissue via PPARγ activation; however, effects within the class differ with regards to histopathologic changes in NASH (21, 22). PPARγ activation (*a*) promotes the differentiation of large, insulin-resistant preadipocytes into small and insulin-sensitive adipocytes; (*b*) reduces inappropriate fat storage in muscle and adipocyte tissue, with subsequent improvement in insulin sensitivity; and (*c*) upregulates production of adiponectin, an insulin-sensitizing and antisteatogenic adipokine that increases fatty acid β-oxidation in liver and muscle (23, 24).

Insulin sensitizers (peroxisome proliferator-activated receptor agonists). PPARs are nuclear receptor proteins that function as transcription factors regulating gene expression. There are three types of PPARs: α (alpha), βδ (beta/delta), and γ (gamma). PPARα is expressed in the liver, intestine, heart, and kidney. Activation of the PPARα receptor promotes uptake, use, and catabolism of fatty acids. It serves as a receptor for fibrates (23). PPARα receptor activation in the liver also has inhibitory activity of inflammatory genes induced by nuclear factor κB (25).

PPARδ is expressed more widely than PPARα and is also involved in lipid accumulation. As a whole, PPAR agonists have been used for a long time in the treatment of diabetes to lower serum triglyceride and glucose levels and make a reasonable compound to study in NASH.

Elafibranor (GT505; GENFIT), a PPAR γδ agonist, has been studied in one Phase 2b trial in individuals with NASH (the GOLDEN-505 trial) (26). A total of 276 patients with biopsy-proven NASH without cirrhosis were treated with 80 or 120 mg/day elafibranor or placebo for 52 weeks. The defined endpoint was improvement in NASH (resolution defined as absence of at least one of the three components of NASH) without a worsening of fibrosis. Initial analysis failed to show any significant difference between the treatment group and placebo: The primary endpoint was achieved in only 23% of patients in the 80 mg/day group, 21% in the 120 mg/day group, and 17% in the placebo group (26). Post hoc analysis with NASH reversal, defined as disappearance of ballooning with either disappearance of lobular inflammation or mild lobular inflammation, reached a statistically significant endpoint in those with a NAFLD activity score (NAS) of 4 or higher at baseline. Additionally, a significant reduction in fibrosis stage was observed in the

Table 1 Current clinical trials in NASH[a]

Agent, company, and https://ClinicalTrials.gov identifier	Phase of trial	Population	Mechanism of action	Target enrollment	Treatment arms	Duration (treatment)	Primary outcome
Obeticholic acid (INT-747) Intercept Pharmaceuticals NCT02548351	3	NASH with fibrosis (stages 2–3)	FXR agonist	2,000	1. Obeticholic acid 10 mg 2. Obeticholic acid 25 mg 3. Placebo	5 years	Improvement of fibrosis of at least one stage and no worsening of NASH NASH resolution without worsening of fibrosis
Elafibranor (GFT505) GENFIT NCT02704403	3	NASH with fibrosis (stages 1–3)	PPAR agonist	2,000	1. Elafibranor 80 mg 2. Elafibranor 120 mg 3. Placebo	5 years	Resolution of NASH without worsening of fibrosis Composite outcome of all-cause mortality, cirrhosis, and liver-related clinical outcomes
Cenicriviroc (CVC) Allergan NCT03028740	3	NASH with fibrosis (stages 2–3)	CCR2/CCR5 antagonist	2,000	1. Cenicriviroc 150 mg 2. Placebo	5 years	Improvement of fibrosis of at least one stage and no worsening of NASH
Selonsertib (GS-4997) Gilead Sciences NCT03053050	3	NASH (with stage 3 fibrosis)	ASK1 inhibitor	800	1. SEL 6 mg 2. SEL 18 mg 3. Placebo	48 weeks	Fibrosis improvement by at least one stage without worsening of NASH
Selonsertib (GS-4997) Gilead Sciences NCT03053063	3	NASH cirrhosis	ASK1 inhibitor	800	1. SEL 6 mg 2. SEL 18 mg 3. Placebo	48 weeks	Fibrosis improvement by at least one stage without worsening of NASH
Emricasan (IDN-6556) Conatus Pharmaceuticals	2	NASH with fibrosis (stages 1–3)	Caspase inhibitor	330	1. Emricasan 5 mg 2. Emricasan 50 mg 3. Placebo	72 weeks	Fibrosis improvement by at least one stage without worsening of steatohepatitis
Volixibat (SHP626) Shire NCT02787304	2	NASH (stages 1–3)	Apical bile salt transporter inhibitor	266	1. SHP626 5 mg 2. SHP626 10 mg 3. SHP626 20 mg 4. Placebo	48 weeks	Reduction in NAS by at least two points without worsening of fibrosis

(*Continued*)

Table 1 (*Continued*)

Agent, company, and https://ClinicalTrials.gov identifier	Phase of trial	Population	Mechanism of action	Target enrollment	Treatment arms	Duration (treatment)	Primary outcome
MSDC 0602K Cirius Therapeutics NCT02784444	2	NASH with fibrosis (stages 1–3), no cirrhosis	Mitochondrial target of thiazolidine-diones (mTOT) modulator	200	1. MSDC-0602K 62.5 mg 2. MSDC-0602K 125 mg 3. MSDC-0602K 250 mg 4. Placebo	1 year	Decrease NAS by two points[b] and no worsening of fibrosis Safety, tolerability, and efficacy
LMB763 Novartis Pharmaceuticals NCT02913105	2	NASH	FXR agonist	100	1. LMB763 2. Placebo	12 weeks	Safety, tolerability, and efficacy Change in transaminases
LJN452 Novartis Pharmaceuticals NCT02855164	2	NASH	FXR agonist	250	1. Multiple dose arms 2. Placebo	12 weeks	Safety, tolerability, and efficacy Change in transaminases
Selonsertib (SEL), GS-0976, and GS-9674 Gilead Sciences NCT02781584	2	Cohorts 1–6: NASH Cohorts 7 and 8: NASH cirrhosis	ASK1 inhibitor ACC inhibitor FXR agonist	110	1. SEL 18 mg 2. GS-0976 (2 × 10 mg) 3. GS-9674 (3 × 10 mg) 4. SEL 18 mg + GS-9674 30 mg 5. SEL 18 mg + GS-0976 20 mg 6. GS-0976 20 mg + GS-9674 30 mg 7. GS-0976 20 mg 8. GS-9674 30 mg	12 weeks	Safety, tolerability, and efficacy
Saroglitazar Zydus Discovery NCT03061721	2	NAFLD/ NASH	PPAR agonist	104	1. Saroglitazar 1 mg 2. Saroglitazar 2 mg 3. Saroglitazar 4 mg 4. Placebo	16 weeks	Percentage change in baseline serum ALT Safety, tolerability, and efficacy

(*Continued*)

Table 1 (*Continued*)

Agent, company, and https://ClinicalTrials.gov identifier	Phase of trial	Population	Mechanism of action	Target enrollment	Treatment arms	Duration (treatment)	Primary outcome
AZD4076 AstraZeneca NCT02605616	2	NAFLD and NASH	Anti-miR-103/107 oligonucleotide	100	1. AZ 4076 800 mg/day 2. Placebo	12 weeks	Change in liver fat fraction Safety, tolerability, and efficacy
NGM282 NGM Bio NCT02443116	2	NASH	FGF19 variant	140	1. Multiple dose arms 2. Placebo	12 weeks	Safety, tolerability, and efficacy Liver fat content
Semaglutide Novo Nordisk NCT02970942	2	NASH	GLP-1 receptor agonist	372	1. Semaglutide 0.1 mg 2. Semaglutide 0.2 mg 3. Semaglutide 0.4 mg 4. Placebo	72 weeks	Resolution of NASH without worsening of fibrosis
Emricasan Conatus Pharmaceuticals NCT02960204	2	NASH cirrhosis and severe portal hypertension	Caspase inhibitor	240	1. Emricasan 5 mg 2. Emricasan 25 mg 3. Emricasan 50 mg 4. Placebo	24 weeks	Mean change in hepatic venous pressure gradient Safety, tolerability, and efficacy
Emricasan Conatus Pharmaceuticals NCT03205345	2	Decompensated NASH cirrhosis	Caspase inhibitor	210	1. Emricasan 25 mg 2. Emricasan 5 mg 3. Placebo	48 weeks	Improvement in event-free survival relative to placebo, based on a composite clinical endpoint

Abbreviations: ALT, alanine aminotransferase; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis.

[a] **https://ClinicalTrials.gov** as of September 2017; US trials Phases 2 and 3 selected for actively recruiting.

[b] Reduction in NAS to include a one-point reduction in ballooning or inflammation; fibrosis evaluated by the NASH Clinical Research Network fibrosis staging system, defined by an increase in at least one stage.

elafibranor 120-mg/day group and improved in those subjects with NASH resolution. Multiple metabolic parameters, including lipid parameters and glucose homeostasis, improved in subjects receiving elafibranor. There was no significant difference in secondary endpoints related to liver histology. Although no major safety signals were identified, a few individuals had a transient increase in serum creatinine. It is currently unclear if this is a PPARα effect, such as that seen with fibrates. This drug is currently being evaluated in a Phase 3 pivotal trial (RESOLVE-IT) (**https://ClinicalTrials.gov** identifier NCT02704403).

Previous treatment for NASH has included the thiazolidinedione class of medications, best exemplified by pioglitazone in the Pioglitazone, Vitamin E or Placebo for Nonalcoholic Steatohepatitis (PIVENS) trial (27). Direct activation of PPARγ is thought to be the mechanism of action of this class of medications; it is also accepted as the main contributor to the side effects of this class of agents (increased number of adipocytes, edema, heart failure, and bone loss). Insulin sensitization independent of PPARγ activation has been shown in knockout mice (28) and carried into a Phase 2 trial (29). Mitochondrial targets of thiazolidinediones (mTOTs) are a current focus of therapeutics in NASH. MSDC-0602K (Octeta Therapeutics) is currently in Phase 2 testing. The primary outcome is histologic improvement (decrease in NAS of at least 2 points, at least 1 point in ballooning or inflammation, with no worsening of fibrosis) at 12 months.

Acetyl-coenzyme A carboxylase inhibitor. Recently discovered effects from acetyl-coenzyme A carboxylase (ACC) inhibition reveal favorable effects on dyslipidemia and hepatic de novo lipogenesis (DNL). ACC plays a key role in regulating fatty acid metabolism. Single doses of NDI-010976, an allosteric inhibitor of ACC1 and ACC2, reduce DNL in overweight and/or obese healthy adult subjects (30). It is currently in Phase 2 testing (NCT02781584).

Incretins. Although they are not currently active clinical trials, antidiabetic drugs in the glucagon-like peptide 1 receptor antagonists (GLP-1RAs) and dipeptidyl peptidase-4 inhibitors are worth mentioning as an emerging area of therapeutic interest. Prevalence estimates of NAFLD in diabetics are as high as 70% (31), and approximately 20% of asymptomatic type 2 diabetics with normal liver function tests have biopsy-proven NASH (32). GLP-1RAs have broad effects on glucose and lipid metabolism. In addition to enhancing insulin secretion, reducing glucagon, and influencing central nervous system effects on appetite, there is evidence that insulin activity in hepatocytes and adipose tissue is improved (33). Multiple animal studies show GLP-1RAs improve hepatic insulin sensitivity and decrease steatosis (34–36). The best evidence for therapeutic efficacy of the GLP-1RAs was offered by the Liraglutide Efficacy and Action in Non-alcoholic steatohepatitis (LEAN) trial. Fifty-two patients with NASH were randomized to receive either subcutaneous injections of liraglutide (1.8 mg daily) or placebo for 48 weeks. Of the patients in the liraglutide arm, 39% met the primary endpoint of resolution of NASH, compared to 9% in the placebo arm [relative risk 4.3 (95% confidence interval 1.0–17.7); $P = 0.019$]. Regarding NASH histology, there was significant improvement in ballooning and steatosis but not in inflammation with liraglutide compared to placebo. Only two patients (9%) treated with liraglutide had progression of fibrosis versus eight (36%) with placebo ($P = 0.04$). Weight loss and improvement in alanine aminotransferase (ALT) were noted in the treatment group (37).

Less robustly studied in NAFLD are the DPP-4 inhibitors, which block the function of DPP-4 that degrades GLP-1. Short-term trials with biochemical endpoints (transaminases) have shown mixed effects. Sitagliptin was associated with improvement in transaminases in one study ($n = 36$) comparing to GLP-1RAs with variable follow-up (38) and with no improvement in transaminases in a slightly larger study ($n = 44$) over 12 months (39). A randomized controlled trial of vildagliptin 50 mg twice daily in 44 patients with diabetes demonstrated reduction in ALT levels (from 27 to

20 IU/L, $P < 0.001$) and intrahepatic triglyceride by proton magnetic resonance spectroscopy (from 7.3% to 5.3%, $P = 0.001$). Insulin sensitivity was not improved (40). Larger randomized controlled trials with histologic endpoints are needed for these agents to draw meaningful therapeutic conclusions.

NASH Agents that Target Bile Acid Metabolism

Farnesoid X receptor agonists. The farnesoid X receptor (FXR) is an orphan nuclear receptor and the cognate receptor for bile acids. Activation of FXR regulates glucose and lipid metabolism. Obeticholic acid (OCA; Intercept Pharmaceuticals), a semisynthetic derivative of chenodeoxycholic acid, is a potent activator of the FXR. OCA has shown activity along several metabolic pathways that are common to NASH, including hepatic steatosis, glucose tolerance, and inflammation (41). It is approximately 100-fold more potent on the FXR than the endogenous chenodeoxycholic acid (42). Of particular interest is its potential role in inhibiting hepatic stellate cell (HSC) activity and potential effects on hepatic fibrosis, demonstrated in animal models (43) and in Phase 2 testing.

In the Phase 2 multicenter Farnesoid X Receptor Ligand OCA in NASH Treatment (FLINT) trial, OCA at a dose of 25 mg daily was compared with placebo in patients with NASH without cirrhosis for 72 weeks. Stratification was done by center and diabetes status. The primary endpoint was an improvement in NAS of at least 2 points without worsening of fibrosis from baseline to the end of treatment. A prespecified interim analysis in the primary outcome demonstrated that OCA was superior to placebo and met criteria for early discontinuation. Liver histology improved (defined by a decrease in NAS of at least 2 points without worsening of fibrosis) in 50 (45%) of 110 patients in the OCA group compared with 23 (21%) of 109 patients receiving placebo. A small improvement in fibrosis was noted in addition to improvement in the NAS components. Adverse events included pruritus in 33 (23%) of 141 patients in the associate group compared with 9 (6%) of 142 in the placebo group. However, there was also a statistically significant increase in low-density lipoprotein cholesterol and a decrease in high-density lipoprotein cholesterol. The clinical significance of these findings remains to be established. OCA is currently being evaluated in a Phase 3 clinical trial (REGENERATE) powered to detect two primary endpoints: fibrosis improvement without worsening of NASH and NASH resolution without worsening of fibrosis (NCT02548351).

Ileal bile acid transporter inhibition. Cholesterol metabolism can also be targeted at the level of the enterohepatic circulation. In blocking bile acid resorption in the terminal ileum, bile acid excretion increases and hepatic bile acid synthesis increases. This is expected to decrease hepatic cholesterol load by diverting it for additional bile acid synthesis. Hepatic free cholesterol is increased in NASH and has been linked to disease severity (44). By reducing hepatic cholesterol, intestinal bile salt reabsorption inhibition is hypothesized to improve NASH. Cellular free cholesterol can induce ER stress and activate inflammatory signaling and apoptosis (45). A Phase 1, double-blind, placebo-controlled trial of 84 overweight or obese men and nonchildbearing women randomized for dose finding of volixibat (SHP626, Shire) versus placebo showed increased bile acid excretion and decreased serum lipids; furthermore, volixibat was minimally absorbed, with adequate safety and tolerability profiles. Phase 2 testing is under way.

NASH Therapies that Target Inflammation

The metabolic perturbation associated with NASH and resultant inflammatory signaling and apoptosis drive macrophage activation, which further amplifies the inflammatory signaling.

Inflammation is a key driver of progressive fibrotic remodeling of the liver as well as oncogenesis. This provides a strong rationale to target inflammation and oxidative stress for therapy (see **Figure 1**). C-C chemokine receptors type 2 and 5 (CCR2/CCR5) are inflammatory chemokines overexpressed in NASH; their ligands are C-C chemokine ligand type 2 [CCL2/monocyte chemotactic protein-1 (MCP-1)] and type 5 [CCL5/regulated on activation, normal T-cell expressed and secreted (RANTES)]. Cenicriviroc (CVC) antagonizes CCR2/CCR5 and has clinical utility in anti-inflammatory and antifibrotic activity after demonstration in animal models (46) and in Phase 2 testing in humans. The ORION trial [Effect of CCR2 and CCR5 Antagonism by Cenicriviroc on Peripheral and Adipose Tissue Insulin Sensitivity in Adult Obese Subjects With Prediabetes or Type 2 Diabetes Mellitus and Suspected Non-Alcoholic Fatty Liver Disease (NAFLD)] included 45 patients with biopsy-proven NAFLD and prediabetes or type 2 diabetes mellitus. Patients were randomized to 150 mg/day or placebo for 24 weeks. The primary outcome of this trial was change in insulin sensitivity from baseline to end of treatment. The Efficacy and Safety Study of Cenicriviroc for the Treatment of NASH in Adult Subjects With Liver Fibrosis (CENTAUR) trial, a Phase 2b study, included noncirrhotic biopsy proven NASH patients with histologic improvement (NAS) without worsening of fibrosis after 1 year or CVC treatment versus placebo as its primary endpoint (47). Following a year of exposure, subjects receiving CVC demonstrated an improvement in fibrosis but did not demonstrate any changes in steatosis or ballooning. This is in line with its mechanism of action, which is at the interface of inflammation and fibrosis.

NASH Therapies that Target Cell Injury

Hepatocellular ballooning and apoptosis are histologic features associated with NASH. Emricasan (Conatus Pharmaceuticals) is a pan-caspase protease inhibitor. Given their role in induction of apoptosis and programmed cell death by cytokine stimulation, caspases complete apoptotic pathways. In preclinical models of NASH, emricasan was shown to inhibit fibrosis, inflammation, and apoptosis. These changes in fibrosis in animal models were associated with reductions in hepatic steatosis but were not accompanied by changes in liver injury (48). Although a Phase 2 study was limited to a study of 38 patients with NAFLD and raised transaminases, emricasan met primary endpoints of significant reductions in markers of inflammation and apoptosis, namely ALT and caspase-cleaved cytokeratin18 (cCK18) levels (49). No toxicities or adverse events were reported (NCT02686762).

NASH Therapies that Target Fibrosis

Many of the metabolic pathways discussed above lead to ongoing liver inflammation and a wound healing response in the liver known as fibrosis and mediated by HSC activation. HSC activation involves molecular signaling pathways that include hedgehog, phosphatidylinositol 3-kinase/protein kinase B, Janus kinase/signal transducer and actuator of transcription, and transforming growth factor-β. This wound healing response is characterized by deposition and extracellular matrix composed of collagen, glycoproteins, glycosaminoglycans, matrix proteins, and growth factors. Although therapeutics targeting the extracellular matrix are of ongoing interest, there are currently no actively enrolling clinical trials targeting fibrosis as a sole primary endpoint in NASH (**Table 1**).

Galectins belong to a family of proteins that bind to terminal galactose residues on glycoproteins through a carbohydrate-binding domain (50, 51). Marked increases in galectin-3 expression during acute or chronic inflammation with resultant fibrogenesis have been observed (52). GR-MD-02 (Galectin Therapeutics), a galactoarabino-rhamnogalacturonan polysaccharide polymer,

functions as a galectin-3 inhibitor. Although the exact mechanism of action remains to be studied, the carbohydrate moieties seem to target macrophages involved in fibrogenesis. Galectin-3 knockout mice demonstrated decreased liver fibrosis after injury, prompting clinical investigation. Preclinical studies of galectin-3 inhibition in a mouse model of NASH showed an association with NASH regression in all parameters, including steatosis, ballooning, and inflammation (53). With a more advanced-disease animal model, GR-MD-02 was shown to reduce fibrosis, reverse cirrhosis, and improve portal hypertension (54), the most important determinant of liver-related outcomes in cirrhosis. Phase 2a testing in patients with advanced fibrosis failed to demonstrate improvements using noninvasive measures of fibrosis (NASH-FX trial; NCT02421094). A Phase 2 trial of GR-MD-02 versus placebo is ongoing but no longer recruiting, with a primary endpoint of reduction of hepatic venous pressure gradient (NASH-CX trial; NCT02462967).

Lysyl oxidase-like 2 (LOXL2) is an enzyme associated with collage cross-linking; expression is greater in fibrotic livers. Although experimental models of NASH treated with a monoclonal antibody to LOXL2 revealed an improvement in fibrosis (55), a Phase 2b randomized, double-blind, placebo-controlled trial of GS-6624 (Gilead Sciences) in NASH with advanced fibrosis and NASH cirrhosis was terminated after it failed to reach its primary endpoints: morphometric quantitative collagen on liver biopsy and event-free survival (in advanced fibrosis; NCT01672866) and mean change in hepatic venous pressure gradient and event-free survival (in cirrhosis; NCT01672879).

Another compound that is under active investigation is selonsertib, which inhibits apoptosis signaling kinase-1 (ASK-1). This is a key target in inflammatory activation related to oxidative stress as well as ER stress. In an early phase trial, selonsertib reduced disease activity and fibrosis stage. These data are now being confirmed in large-scale trials in those with NASH and bridging fibrosis or cirrhosis (NCT03053050 and NCT03053063).

THE FUTURE OF NASH THERAPEUTICS

Despite the development of multiple therapies for NASH, there is no shortage of additional targets to be found. Risk factors for NASH overlap those of other common complex diseases such as obesity and diabetes, but the common pathways within the liver may overlap in a profibrotic mechanism that has yet to be determined. To better understand the biology of such a heterogeneous disease, phenotypic variation will need further exploration, and therapies will likely be targeted along particular phenotypes based on overall mortality risk. Importantly, phenotyping should include cardiometabolic profiling, given disease-related outcomes of cardiovascular endpoints. Dual or even triple therapy may be required to limit fibrosis. Of critical interest is the prevention of liver disease–related outcomes: decompensation, death, the need for transplantation, and/or development of hepatocellular carcinoma. As therapies for the underlying metabolic and inflammatory pathways promoting disease activity advance and antifibrotic therapies are developed, it is critically important that similar efforts with regards to disease prevention should also be undertaken.

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