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Review

Current and Emerging Approaches for Nonalcoholic Steatohepatitis Treatment

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Nonalcoholic steatohepatitis (NASH) is the second leading cause of liver transplantation in the US with a high risk of liver-related morbidities and mortality. Given the global burden of NASH, development of appropriate therapeutic strategies is an important clinical need. Where applicable, lifestyle modification remains the primary recommendation for the treatment of NASH, even though such changes are difficult to sustain and even insufficient to cure NASH. Bariatric surgery resolves NASH in such patients where lifestyle modifications have failed, and is recommended for morbidly obese patients with NASH. Thus, pharmacotherapies are of high value for NASH treatment. Though no drug has been approved by the US Food and Drug Administration for treatment of NASH, substantial progress in pharmacological development has been made in the last few years. Agents such as vitamin E and pioglitazone are recommended in patients with NASH, and yet concerns about their side effects remain. Many agents targeting various vital molecules and pathways, including those impacting metabolic perturbations, inflammatory cascades, and oxidative stress, are in clinical trials for the treatment of NASH. Some agents have shown promising results in phase II or III clinical trials, but more studies are required to assess their long-term effects. Herein, we review the potential strategies and challenges in therapeutic approaches to treating NASH.

Key words: Nonalcoholic steatohepatitis (NASH); Pharmacological therapy; Molecular targets; Lifestyle modification; Bariatric surgery

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), which is a clinicopathological spectra of liver diseases including simple steatosis, nonalcoholic steatohepatitis (NASH), and cirrhosis, has become a major cause of chronic liver disease in adults[1]. NASH is characterized by steatosis, lobular inflammation, and hepatocellular ballooning and can potentially progress to cirrhosis[2,3]. It is the second leading cause of liver transplantation in the US with a high risk of liver-related morbidity and mortality[4,5]. At present, the global prevalence rate of NAFLD is approximately 25%, while the prevalence of NASH in general people is estimated to be 3%–5% worldwide[6,7]. NASH is highly related to obesity, dyslipidemia, and type 2 diabetes[7]. Due to the growing prevalence of obesity, the incidence of NASH may increase further. The high incidence and the poor clinical outcomes of NASH thus are being viewed as a serious global burden.

No US Food and Drug Administration (FDA)-approved medication currently exists for NASH[8]. Some available strategies, including lifestyle modification, bariatric surgery, and pharmacological therapies, have been recommended for the treatment of NASH in eligible and appropriate patients. The currently recommended treatments have their limitations, so new medical treatments are needed. To date, several novel therapies targeting different stages of NASH pathogenesis, including metabolic perturbations, inflammatory cascades, and oxidative stress, have entered clinical trials (Fig. 1). In addition, it is

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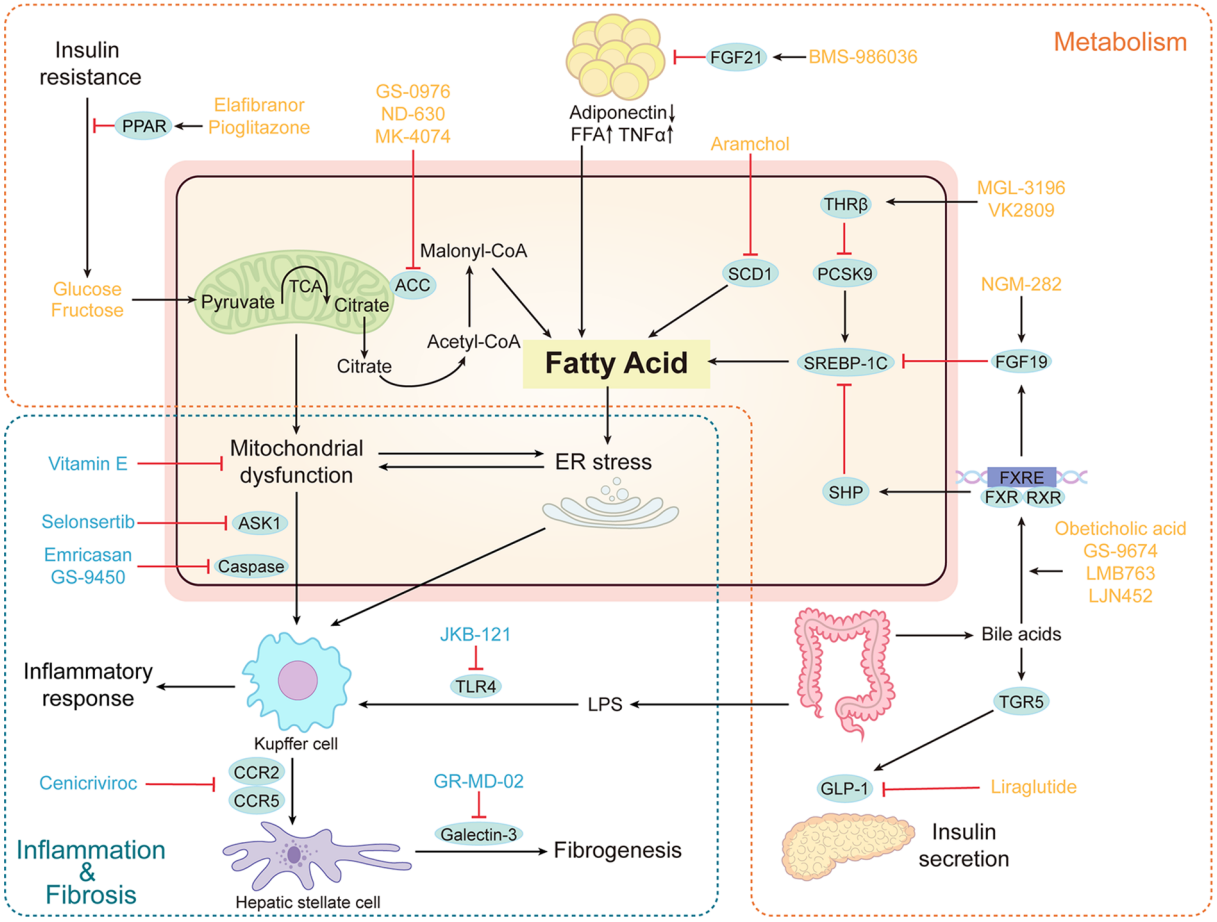

Figure 1. Core mechanisms of therapeutic targets for nonalcoholic steatohepatitis (NASH). (1) Agents that target metabolic homeostasis in the liver. This group includes ACC inhibitors, FXR agonists, FGF 19/21 analog, PPAR agonist, THRβ agonists, SCD1 inhibitor, GLP-1 receptor agonist. (2) Agents that target inflammatory cascade and oxidative stress. This group includes vitamin E, CCR2/5 antagonist, TLR4 antagonist, ASK1 inhibition, galectin-3 inhibitor, and caspase inhibitor. Some therapeutic pathways (orange box) broadly regulate metabolic pathways. Other therapeutic pathways (blue box) regulate inflammatory cascade and oxidative stress. The blue circles indicate therapeutic targets. PPAR, peroxisome proliferator-activated receptor; TCA, tricarboxylic acid; ACC, acetyl-CoA carboxylase; FFA, free fatty acid; SCD1, stearyl coenzyme a desaturase 1; TNFα, tumor necrosis factor α; FGF21, fibroblast growth factor 21; SREBP, sterol regulatory element-binding proteins; PCSK9, proprotein convertase subtilisin/kexin 9; THRβ, thyroid hormone receptor β; FGF19, fibroblast growth factor 19; SHP, small heterodimer partner; FXR, farnesoid X receptor; RXR, retinoid-x receptor; FXRE, farnesoid X receptor element; TGR5, Takeda G protein-coupled receptor 5; GLP-1, glucagon-like peptide 1; ASK1, apoptosis signal-regulating kinase 1; CCR, C-C motif chemokine receptor; TLR4, toll-like receptor 4; LPS, lipopolysaccharide.

exciting that animal studies along with subsequent clinical trials have shown some promising results. This review discusses up-to-date therapies that have been developed to treat NASH and are mainly in phase II or III trials and their pharmacologic targets, in hopes of bringing new insights into the mechanisms and treatment of NASH.

CURRENTLY RECOMMENDED THERAPIES

Lifestyle Modification

Lifestyle modification has represented the cornerstone of therapies for patients with NASH and includes weight loss, diet, and exercise. A comprehensive lifestyle intervention that includes reduced calorie intake and increased exercise can improve the histological features of NASH[9].

Weight Loss

Modest weight loss reduced inflammation and altered homeostasis in humans, shedding light on its potential value for treating NASH[10]. The amount of weight loss is a determining factor in improving histological features of NASH patients. Specifically, weight reduction of 3%–5% is helpful for improving steatosis, while reductions of 5%–7% are necessary for attenuating inflammation[11,12].

Significant weight loss of 7% or more may improve the histologic features of NASH, involving hepatic steatosis, ballooning, and lobular inflammation[13–15], while weight reductions of ≥10% are required to improve fibrosis and portal inflammation, as exhibited in a prospective trial[11]. Unfortunately, more than half of the subjects fail to achieve the goal of losing 7% of body weight at 12 months in the same study, underscoring the challenges of lifestyle modifications as a universal and practical intervention for NASH.

Diet

A dietary fat with saturated fatty acids is associated with the development of NASH, but $n-3$ polyunsaturated fatty acids have protective effects on NASH by mitigating hepatic oxidative stress and inflammation[16,17]. Recently, the Mediterranean diet, identified as a low-fat and restricted-calorie diet with ample $n-3$ polyunsaturated fatty acids, has been demonstrated to reduce hepatic fat and improve hepatic insulin sensitivity, and adherence to the Mediterranean diet lower the risk for NASH. Moreover, this diet is recommended by the EASL-EASD-EASO Clinical Practice Guidelines[18–20]. Fructose intake should be limited in patients with NASH because its consumption was associated with NASH[21]. In addition, some dietary supplements contribute to the treatment of NASH, such as coffee and probiotics[22–24].

Exercise

Exercise is beneficial for improving liver fat and histology of NASH[25,26]. Different forms of exercise have similar beneficial effects on hepatic fat, and vigorous exercise has the largest effects on NASH and fibrosis[9]. However, these effects disappear if patients do not exercise persistently. Vigorous exercise of no less than 250 min/week mitigates the pathophysiology of NASH better than exercise of less than 250 min/week, but excessive exercise is harmful for patients with cardiovascular complications[12]. Moderate intensity aerobic exercise over three to five sessions for 150–200 min/week is recommended by the EASL-EASD-EASO guidelines[18]. More studies on exercise are needed to verify its long-term effects on NASH and the related morbidity and mortality.

Although lifestyle modifications are considered generally safe, rapid weight loss achieved by any modality may increase the risk of cholelithiasis because of the increase in cholesterol flux through the biliary system, and many patients may experience problems regarding steadfast adherence[27].

Bariatric Surgery

Bariatric surgery is an effective way to obtain long-term weight loss. Bariatric surgery prevented NASH progression in diet-induced obese rats of an animal research[28], and the current evidence has demonstrated that liver steatosis, inflammation, and fibrosis were improved in NASH patients after bariatric surgery[29,30]. What is more, a recent study indicated that bariatric surgery has a long-term effect of mitigating NASH, and the effect could last for 10 years after various bariatric surgeries[31]. Bariatric surgery is applicable in morbidly obese patients with NASH and post-liver transplantation recurrent NASH[32]. However, insufficient clinical data exist to recommend bariatric surgery as a routine treatment for NASH considering the perioperative risks and cost effectiveness[29]. Larger randomized controlled trials (RCT) should be designed to confirm the efficacy and safety of bariatric surgery on NASH.

Pharmacological Therapy

Pharmacotherapies should be considered for NASH and fibrosis patients due to the limitations of lifestyle modification and bariatric surgery. Vitamin E and pioglitazone have been suggested as pharmacotherapies for histologically confirmed NASH patients in guidelines from the US, Europe, China, and Japan.

Vitamin E

Currently, vitamin E is the accepted first-line pharmacological treatment for NASH in nondiabetic patients. Vitamin E is an essential antioxidant that acts by inhibiting lipid peroxidation and improving lipid metabolism. In mouse models of NASH, vitamin E attenuated lipid peroxidation and inflammatory responses[33]. The potential effect of vitamin E on NASH has been evaluated in four clinical trials of which the PIVENS trial (Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis) is the largest RCT[34–37]. In the PIVENS trial, 247 subjects with histologically confirmed NASH were randomly assigned to receive pioglitazone 30 mg/day (80 patients), vitamin E 800 IU/day (84 patients), or placebo (83 patients) for 96 weeks[37]. Vitamin E was superior to the placebo in improving alanine transaminase (ALT) levels, liver steatosis, and inflammation but did not significantly mitigate fibrosis. The long-term safety of vitamin E has been controversial, so its benefits need to be weighed against the increased risks of prostate cancer, hemorrhagic stroke, and even all-cause mortality[38].

Pioglitazone

Peroxisome proliferator-activated receptors (PPARs), including PPARα, δ, and γ, are nuclear receptors involved in lipid metabolism. Pioglitazone, a PPARγ agonist, regulates plasma adiponectin and improves insulin sensitivity in the liver[39]. It could improve histologic alterations including hepatic injury and fibrosis in patients with NASH[40]. The function of alleviating histological components was

also verified in the PIVENS trial, but this efficacy did not reach the predicted threshold[37]. It did resolve steatohepatitis in a subset of patients. A similar long-term RCT involving 101 prediabetic and diabetic patients with biopsy-proven NASH showed that pioglitazone treatment improved all histologic features and ameliorated NASH without a worsening of fibrosis[41]. Pioglitazone should be used carefully for NASH in diabetic patients with potential long-term risk concerns.

Some drugs such as statins, metformin, and lipase inhibitors seem to be safe but are not recommended because no histological data are available to support their effects on NASH. More novel pharmacological approaches are necessary for treating NASH because the long-term safety and impact on currently recommended pharmacotherapies should be further evaluated.

PROMISING MOLECULAR TARGETS FOR PHARMACOLOGICAL THERAPY

Some pharmacological therapies with different targets, including those impacting metabolic perturbations, inflammatory cascades, and oxidative stress, and are in phase II or III trials are promising for the treatment of NASH. Recently completed and ongoing RCTs for NASH are summarized in Table 1.

Targeting Metabolic Perturbations

Acetyl-CoA Carboxylase (ACC) Inhibitors. ACC regulates the nonreversible conversion of acetyl-CoA to malonyl-CoA and includes the isoforms ACC1 and ACC2[42]. Malonyl-CoA regulates fatty acid synthesis, which is a hallmark of various metabolic diseases including NASH. At present, several ACC inhibitors have shown beneficial effects in preclinical studies and clinical trials of NASH. Notably, GS-0976, an inhibitor of ACC1 and ACC2, decreased liver steatosis, markers of liver injury, and de novo lipogenesis (DNL) in a placebo-controlled trial of NASH patients[43]. ND-630, an isozyme-nonselective ACC inhibitor, can reduce fatty acid synthesis and promote fatty acid oxidation in cultured cells and animals. In animal models, ND-630 decreased liver steatosis, improved insulin sensitivity, as well as modulated dyslipidemia[44]. However, no clinical trial has tested the effect and safety of ND-630 in NASH patients. MK-4074, a liver-targeting inhibitor of ACC1 and ACC2, reduced hepatic triglyceride (TG) levels in preclinical studies and significantly reduced liver fat in human beings[45]. Unfortunately, the use of ACC inhibitors can increase the expression of glycerol-3-phosphate acyltransferase 1, which plays an important part in mediating triglyceride synthesis, ultimately leading to significant hypertriglyceridemia[45].

Farnesoid X Receptor (FXR) Agonists. FXR was initially identified as a bile acid sensor that controls bile acid and lipid homeostasis through the small heterodimer partner (SHP)[46,47]. Obeticholic acid (OCA), an agonist of FXR, improves insulin sensitivity, inflammation, and fibrosis and reduces hepatic steatosis in obese rats[48]. In a recent clinical trial (FLINT), OCA induced a definite improvement in NASH activity, serum aminotransferase, and fibrosis score compared with placebo[49]. Furthermore, in a phase II study, OCA administered to NASH patients for 6 weeks increased insulin sensitivity and attenuated hepatic inflammation and fibrosis[50]. Two phase III trials involving 3,000 patients from more than 20 countries are upcoming to verify its influence (ClinicalTrials.gov Identifier: NCT02548351, NCT03439254). As exhibited in the interim results of phase III trials, OCA improved hepatic fibrosis with no worsening of NASH compared with placebo. However, OCA has several side effects, like pruritus, gastrointestinal events, and so on[49]. A nonsteroidal FXR agonist, GS-9674, has been exploited to address some of the side effects of OCA. GS-9674 mitigated liver steatosis and hepatic biochemistry in subjects with NASH in a phase II RCT (ClinicalTrials.gov Identifier: NCT02854605). Other FXR agonists, such as LMB763 and LJN452, with limited side effects, have also been tested in phase II trials (ClinicalTrials.gov Identifier: NCT02913105, NCT02855164).

Fibroblast Growth Factor 19/21 (FGF 19/21) Analog. A peptide hormone FGF19 is released from the intestine after bile acid binding to FXR and regulates bile acid synthesis and lipid metabolism[51,52]. In a preclinical study, treatment with FGF19 significantly decreased diet-induced hepatic steatosis[53]. NGM-282 is a recombinant FGF19 with the beneficial metabolic effects of reducing steatosis and lipotoxicity. Furthermore, a phase II study of 166 biopsy-confirmed NASH patients indicated that NGM-282 significantly reduced liver fat content with an acceptable safety profile as well as adverse events, including diarrhea, abdominal pain, and nausea[54]. Another phase II multiple-center study is ongoing to ascertain the safety and effectiveness of NGM282 in histologically confirmed NASH patients (ClinicalTrials.gov Identifier: NCT02443116).

FGF21, a 181-amino acid-secreted protein produced in the liver, enhances glycogen synthesis and ameliorates insulin sensitivity, which was beneficial in treating NASH in rodent models[55–57]. Due to the short half-life period of wild-type FGF21, the recombinant mutant FGF21, and the conjugation of FGF21 to polyethylene glycol (PEG), has been developed and was tested for its role in NASH. This variant of FGF21 (LY2405319) alleviated inflammation and reversed hepatofibrosis in an animal study of NASH[58]. Notably, a PEGylated FGF21 (BMS-986036) was demonstrated to significantly reduce hepatic fat in NASH patients and had good tolerance in a phase II clinical study[59]. Two phase II trials of BMS-986036 in NASH patients with stage 3 disease are currently

Table 1. Recently Completed and Ongoing Randomized Controlled Trials for NASH.

Agents	Mechanisms	Primary Endpoint(s)	Phase Completed	Patients (*n*)	Duration	Status	NCT
GS-0976	ACC inhibitor	Overall safety	II	125	Up to12 weeks plus 30 days	Completed	NCT02856555
Obeticholic acid	FXR agonist	Improvement in liver histology in non-cirrhotic NASH	III	2500	18 months	Recruiting	NCT02548351
Obeticholic acid	FXR agonist	Improvement in fibrosis	III	540	12 months	Recruiting	NCT03439254
GS-9674	FXR agonist	Overall safety	II	125	Up to 24 weeks plus 30 days	Completed	NCT02854605
LMB763	FXR agonist	Adverse event profile and safety; change in transaminase levels	II	100	12 weeks	Recruiting	NCT02913105
LJN452	FXR agonist	Adverse event profile; change in transaminase levels and fat in the liver	II	250	12 weeks	Recruiting	NCT02855164
NGM-282	FGF19 analog	Change in hepatic fat	II	75	24 weeks	Recruiting	NCT02443116
BMS-986036	FGF 21 analog	Improvement in fibrosis	II	160	24 weeks	Recruiting	NCT03486899
BMS-986036	FGF 21 analog	Improvement in fibrosis	II	100	48 weeks	Recruiting	NCT03486912
Elafibranor	PPARα/δ agonist	Resolution of NASH without worsening fibrosis	III	2000	72 weeks	Recruiting	NCT02704403
MGL-3196	THRβ agonist	Change in hepatic fat	II	117	12 weeks	Active, not recruiting	NCT02912260
Aramchol	SCD-1 inhibitor	Change in the hepatic fat	II	240	52 weeks	Completed	NCT02279524
Liraglutide	GLP-1 receptor agonist	Improvement in NASH	III	36	12 months	Recruiting	NCT02654665
Cenicriviroc	CCR2/5 antagonist	Improvement in liver histology	III	2000	12 months	Recruiting	NCT03028740
JKB-121	TLR4 antagonist	Change in hepatic fat and improvement in ALT	II	60	24 weeks	Completed	NCT02442687
GS-4997 + Simtuzumab	ASK1 inhibitor, LOXL2 inhibitor	Adverse event profile	II	70	Up to 28 weeks	Completed	NCT02466516
Selonsertib	ASK1 inhibitor	Improvement in fibrosis without worsening NASH	III	800	48 weeks	Active, not recruiting	NCT03053050
GR-MD-02	Galectin-3 inhibitor	Improvement in fibrosis	II	30	16 weeks	Completed	NCT02421094
Emricasan	Caspase inhibitor	Improvement in fibrosis without worsening steatohepatitis	II	330	72 weeks	Active, not recruiting	NCT02686762
GS-9450	Caspase inhibitor	Safety and tolerability	II	110	8 weeks	Completed	NCT00740610

ACC, acetyl-CoA carboxylase; FXR, farnesoid X receptor; FGF19, fibroblast growth factor 19; FGF21, fibroblast growth factor 21; PPARα/δ, peroxisome proliferator-activated receptor α/δ; THRβ, thyroid hormone receptor β; LDL-C, low-density lipoprotein cholesterol; SCD1, stearyl coenzyme a desaturase 1; GLP-1, glucagon-like peptide 1; CCR2/5, C-C motif chemokine receptor 2/5; TLR4, toll-like receptor 4; ALT, alanine transaminase; ASK1, apoptosis signal-regulating kinase 1; LOXL 2, lysyl oxidase-like protein 2.

ongoing (ClinicalTrials.gov Identifier: NCT03486899, NCT03486912).

PPARα/δ Agonist. PPARα activation regulates fatty acid β-oxidation and lipid transport, while PPARδ activation results in fatty acid oxidation[60]. Elafibranor (GFT505) is a dual PPARα/δ agonist that can improve peripheral insulin sensitivity. Treatment of an animal model of NASH demonstrated a decrease in hepatic fat and improvement in hepatic steatosis, inflammation, and fibrosis[61]. The antifibrotic effect of GFT-505 was independent of metabolic corrections. Elafibranor also reduced liver enzyme concentrations and had a reassuring safety profile[62]. Recent results from the phase IIb RCT demonstrated that Elafibranor resolved NASH and lowered ALT levels without worsening of fibrosis. It also exhibits beneficial effects on metabolic markers, including improving insulin sensitivity, lowering plasma triglycerides, and increasing high-density lipoprotein cholesterol[63]. Furthermore, it was well tolerated and thus may be a promising drug candidate for NASH therapy[63]. A pivotal phase III RESOLVE-IT trial of Elafibranor is currently recruiting patients (ClinicalTrials.gov Identifier: NCT02704403).

Thyroid Hormone Receptor β (THRβ) Agonists. THRβ is the crucial nuclear receptor for the action of thyroid hormones, especially triiodothyronine (T3), on hepatocytes. It has been confirmed to reduce hepatic lipotoxicity and improve hepatic function by lowering fatty acid accumulation and degeneration[64]. THRβ agonists have also been shown to induce liver regeneration via stimulation of Wnt/β-catenin pathway and inducing hepatocyte proliferation, while reducing hepatocellular cancer burden, which could all be beneficial in NASH[65–67]. A THRβ agonist prevented hepatic steatosis but impaired insulin sensitivity in high-fat diet-fed rats[68]. In a multicenter study in adults with biopsy-confirmed NASH, a highly selective liver-targeted THRβ agonist MGL-3196 for 12 weeks significantly decreased hepatic fat relative to placebo[69]. Moreover, VK2809, a liver-targeted THRβ agonist, was shown to reduce liver triglycerides and microvesicular steatosis in rats and mice[70]. Phase II trials of MGL-3196 and VK2809 are scheduled to begin enrolling patients soon (ClinicalTrials.gov Identifier: NCT02912260, NCT02927184).

Stearoyl Coenzyme A Desaturase 1 (SCD1) Inhibitor. SCD1 is the central enzyme that catalyzes the synthesis of fatty acids and decreases β-oxidation[71]. Aramchol, a novel SCD1 inhibitor, reduces hepatic fat content and mitigates steatohepatitis and fibrosis in mice[72]. In a phase II RCT of biopsy-proven NASH and NAFLD subjects, Aramchol given once a day for 3 months reduced hepatic fat with no adverse events[73]. However, this trial did not verify the effect of Aramchol on hepatic inflammation and fibrosis associated with NASH. In a 52-week phase IIb RCT of biopsy-proven NASH patients, two doses of Aramchol (400 and 600 mg) significantly reduced hepatic fat and improved histologic features with excellent safety and tolerability (ClinicalTrials.gov Identifier: NCT02279524). A phase III trial with a large sample size should be designed to determine the therapeutic effects of Aramchol.

Glucagon-Like Peptide 1 (GLP-1) Receptor Agonist. GLP-1, first described as a gut-derived hormone generated through the proteolytic processing of proglucagon[74,75], has an influence on liver metabolism in NASH[76,77]. Preclinical studies observed reductions in liver enzymes and oxidative stress accompanied by improvements in liver histology in murine models of NASH after GLP-1 receptor agonist intervention[78,79]. The GLP-1 receptor agonist liraglutide was reported to ameliorate hepatic inflammation and ballooning in an animal study[80]. In humans, a randomized, placebo-controlled phase II trial showed liraglutide to cause histological resolution of NASH without deterioration of fibrosis[81]. It was found to be safe and well tolerated, but extensive, longer duration studies are warranted[81,82]. A phase III study to compare the therapeutic potency of liraglutide and bariatric surgery in NASH is currently enrolling cases (ClinicalTrials.gov Identifier: NCT02654665).

Targeting Inflammation and Oxidative Stress

C-C Motif Chemokine Receptor 2/5 (CCR2/5) Antagonist. The CCR2/5 chemokine axis recruits immune cells to the liver to initiate an immune response[83]. The CCR2/5 antagonist Cenicriviroc (CVC) displays anti-inflammatory and antifibrotic activities in preclinical models and may be useful in the treatment of NASH[84]. Presently, its efficacy and safety are being tested in a phase IIb RCT (CENTAUR trial) for treating NASH and hepatic fibrosis. CVC has shown a significant antifibrotic benefit with no worsening of steatohepatitis after 1 year. Compared with placebo, biomarkers of inflammation were significantly reduced after CVC intervention[85]. A phase III study is currently recruiting patients and evaluating the role of CVC for the treatment of NASH (ClinicalTrials.gov Identifier: NCT03028740).

Toll-Like Receptor 4 (TLR4) Antagonist. TLR4 is a crucial mediator of innate immunity and is involved in inflammation and insulin resistance[86,87]. It has been a potential target for amelioration of hepatic injury, insulin resistance, and progression to NASH in preclinical studies[88,89]. Notably, the TLR4 antagonist JKB-121 reduced LPS-induced inflammatory liver injury and inhibited hepatic stellate cell activation, supporting JKB-121 as a potential treatment for NASH. However, a phase II RCT of JKB-121 in 65 patients with NASH was completed with unsatisfactory results. Compared to placebo, JKB-121 did not further improve liver fat or ALT and

showed mild drug-related adverse events (ClinicalTrials.gov Identifier: NCT02442687).

Apoptosis Signal-Regulating Kinase 1 (ASK-1) Inhibitor. ASK1 is a member of the MAP3K family and regulates the p38/JNK pathways[90]. ASK1 inhibition improves liver steatosis, inflammation, insulin resistance, and fibrosis in rodent and primate models of NASH[91–93], suggesting CASP8, FADD-like apoptosis regulator (CFLAR), and TNF-α-induced protein 3 (TNFAIP3) as potential targets for the treatment of NASH[94,95]. Selonsertib (GS-4997), which targets ASK1, was assessed in a multicenter phase II trial in which selonsertib was shown to alleviate hepatic fibrosis in NASH patients and those with stage 2 or 3 fibrosis[96]. Therapy using a combination of selonsertib and simtuzumab improved fibrosis with high-dose selonsertib (ClinicalTrials.gov Identifier: NCT02466516). In addition, patient-reported outcomes displayed that selonsertib or selonsertib in combination with simtuzumab lowered hepatic collagen in NASH patients and stages 2–3 liver fibrosis[97]. Selonsertib is now being tested in a phase III trial for the treatment of NASH and F3 fibrosis (ClinicalTrials.gov Identifier: NCT03053050). Unfortunately, the latest report by Gilead shows that selonsertib failed to achieve the main goal of improving liver fibrosis in the phase III trial.

TGF-β-Activated Kinase 1 (TAK1) Inhibitor. TAK1 facilitates the activation of downstream JNK and NF-κB cascades, promoting the development of NASH[87]. Inhibition of TAK1 activity by deubiquitination or dephosphorylation protected against hepatic steatosis, insulin resistance, and inflammation and is thereby considered to be a therapeutic target for NASH[98–101]. In animal studies of NASH, specific proteins targeting TAK1, such as cylindromatosis (CYLD), ubiquitin-specific protease 4 (USP4), and dual-specificity phosphatase 14 (DUSP14), have been shown to hinder the progress of NASH[101–103]. However, no clinical trials have been designed to verify the effects of TAK1 inhibition on NASH.

Galectin-3 Inhibitor. Galectin-3, a β-galactoside-binding animal lectin, is expressed predominantly in immune cells, which regulates the progression of hepatic fibrosis[104]. Galactoarabinorhamnogalacturnate (GR-MD-02), a complex carbohydrate-based inhibitor that binds to galectin-3, ameliorated histopathological features of NASH and fibrosis in a preclinical study of NASH[105]. Furthermore, GR-MD-02 was safe and well tolerated, as demonstrated by the phase I human study of subjects with histologically confirmed NASH and stage 3 fibrosis[106]. Recently, one phase II randomized trial has already completed testing the role of GR-MD-02 in treating NASH patients. However, the results showed no apparent improvement in the levels of noninvasive biomarkers of hepatic inflammation or fibrosis after 16 weeks of therapy with GR-MD-02 (ClinicalTrials.gov Identifier: NCT02421094).

Caspase Inhibitor. Caspases are a class of enzymes that play a critical role in executing apoptotic pathways or programmed cell death. Emricasan (IDN-6556), a currently available irreversible pancaspase inhibitor, has shown an influence on improving NAS and fibrosis in preclinical trials[107]. In a phase II study, 38 subjects with proven NAFLD/NASH were randomly assigned to receive Emricasan 25 mg twice a day or placebo for 28 days with a primary result of reduction in ALT and biomarkers[108]. Recently, a phase IIb study of Emricasan will begin enrolling cases soon (ClinicalTrials.gov Identifier: NCT02686762). Another caspase inhibitor, GS-9450, can act selectively on caspases 1, 8, and 9. As exhibited in a phase II, double-blind trial, GS-9450 significantly reduced ALT levels in patients with NASH[109]. The safety and efficacy of GS-9450 for NASH need to be assessed in larger-scale clinical trials.

CHALLENGES IMPEDING TRANSLATION FROM BENCH TO BEDSIDE IN DRUG DISCOVERY FOR NASH

Drug development for NASH requires clear mechanisms, appropriate animal models, progressive clinical trials, convenient efficacy evaluation, and follow-up methods. Many challenges impeding the translation from bench to bedside remain in this drug discovery process. The pathogenesis of NASH has not been entirely elucidated and has been debated for a long time, which is one of the bottlenecks in drug development. The "multiple-parallel hit" hypothesis was recently proposed, providing a more adequate explanation of how fatty acids and their metabolites promote NASH through multiple sequential or parallel cytotoxic pathways[110]. The pathogenesis of human NASH involves varied molecular pathways and complex progression with a dynamic bidirectional nature, which is unlikely to be the same in all patients, raising concern about individual differences.

The perfect animal model that mimics the pathophysiology of NASH and displays the most clinical characteristics of human disease as closely as possible does not exist, further increasing the difficulty in identifying and validating potential drug targets for human NASH. Many species, including mice, rabbits, pigs, and monkeys, are used to develop models with a liver phenotype resembling human NASH, and each has its advantages and disadvantages[111]. Recently, monkeys have been recommended as models of NASH due to similarities in liver anatomy, physiology, metabolism, and genetics to humans[112,113]. Monkey breeding has specific disadvantages including sophisticated genetic methods, housing, cost, and logistics. Moreover, the ideal pharmacodynamics of drugs

in animal models do not necessarily replicate in human NASH.

Because of the slow progression to clinically significant outcomes in NASH, drug development has been delayed, and the potential time to market for drugs has been extended. The use of optimal surrogate endpoints for clinical trials in NASH is imperative for evaluating pharmacologic agents[114]. For purposes of accelerated approval, the surrogate endpoints used by the FDA can be achieved in a reasonably short timeframe. Biomarkers of NASH are helpful for the diagnosis, monitoring, and prognosis of disease progression and evaluation of the effects of new regimens, which is urgent in the field of NASH. In addition, existing methods of pharmacodynamic evaluation and follow-up are still insufficient to assess NASH progression and regression for drug development.

PROSPECTIVE AND FUTURE

Currently, no FDA-approved drug is available to treat NASH. Lifestyle modification rarely leads to the resolution of NASH, while bariatric surgery can resolve NASH but is just recommended for morbidly obese patients with NASH. Many efforts have been made to develop pharmacotherapies based on the core mechanism of NASH, and some agents have entered phase III favorably. Encouragingly, promising results have been obtained for some medications, including FXR agonists, PPAR-α/δ agonists, GLP-1 agonists, CCR2/5 chemokine receptor antagonists, ASK-1 inhibitors, galectin-3 inhibitors, and caspase inhibitors. However, these drugs are still investigative and experimental. None of these experimental medicines has a perfect function of improving all histological features of NASH and reversing NASH with no drug-related adverse events in the existing clinical trials. Long-term effects and safety on NASH and liver-related mortality and morbidity should be tested in more clinical trials, particularly given concerns about the potential adverse events of some drugs. In the coming years, it is expected that some of these agents alone or in combination will provide the optimal outcomes and new treatment options for NASH patients. Furthermore, improved animal models and scientific research are needed to identify the complete pathogenesis and find new targets for therapy, while specific markers, optimized clinical trial design, suitable surrogate endpoints, and follow-up investigation are required for further drug development.

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