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Review

Emerging Molecular Targets for Treatment of Nonalcoholic Fatty Liver Disease

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In parallel with the obesity epidemic, nonalcoholic fatty liver disease (NAFLD) has emerged as the most common chronic liver disease worldwide. Disequilibrium of lipid metabolism and the subsequent metabolic-stress-induced inflammation are believed to be central in the pathogenesis of NAFLD. Of note, metabolic inflammation is primarily mediated by innate immune signaling, which is increasingly recognized as a driving force in NAFLD progression. Currently, a series of agents targeting one or more of these pathomechanisms have shown encouraging results in preclinical models and clinical trials. This review summarizes the emerging molecular targets involved in signaling in the lipid metabolism and innate immunity aspects of NAFLD, focusing on their mechanistic roles and translational potentials.

Highlights

NALFD is becoming a global epidemic with serious hepatic and extrahepatic complications, but no approved therapeutic drugs are available.

Lipid metabolic perturbation and metabolic-stress-induced inflammation form the core of the pathogenesis of NAFLD.

Innate immune signaling is a key regulator in metabolic inflammation, acting as a driving force in NAFLD progression.

Several therapeutic targets regulating the lipid metabolism and innate immunity of NAFLD have been identified and have shown promising results in clinical trials.

Systems-based multiomic analyses and big data technology, combined with advances in disease models, will be useful for discovering potential therapeutic targets, accelerating the translation from basic to clinic, and providing individualized therapy.

NAFLD Epidemic and Urgent Need for Therapeutic Drugs

NAFLD is a clinicopathological entity that encompasses a wide range of liver disease spectra ranging from steatosis and nonalcoholic steatohepatitis (NASH) to fibrosis and cirrhosis [1]. NAFLD is associated with higher risks of hepatocellular carcinoma (HCC) and severe extrahepatic diseases such as cardiovascular disease (CVD) [2,3]. In parallel with the epidemics of obesity, type 2 diabetes mellitus (T2DM), and metabolic syndrome, NAFLD has become the most common chronic liver disease worldwide and places a tremendous burden on public health [4]. The global prevalence of NAFLD is estimated to be approximately 25%, and it has grown tremendously in recent decades [1]. A recent meta-analysis showed an unexpected rapid increase in the burden of NAFLD in China over the past 10 years, with a national prevalence of 29.2% [5]. In the US, NASH is estimated to be the leading etiology for liver transplantation in the near future [4]. Thus, we are now facing remarkable challenges in dealing with the soaring epidemic of NAFLD worldwide [6,7]. Although the burden of NAFLD is increasing rapidly, unfortunately, there are no FDA-approved effective drugs thus far. Lifestyle modifications via diet and exercise are still the most highly recommended NAFLD treatment, which is hard to sustain for the long term [1]. Therefore, the need to develop effective pharmacological interventions is urgent and imperative.

Lipid Metabolism and Innate Immunity in NAFLD

Over the past few years, countless studies aimed at unraveling the pathogenesis of this disease have been conducted and provided potential therapeutic options [8,9]. Mounting evidence has demonstrated that the development of hepatic steatosis in NAFLD may result from an imbalance between lipid production and breakdown due to an overwhelmed liver metabolic capacity. Excessive accumulation of lipids induces distinct liver metabolic stress and subsequently causes lipotoxicity, which triggers the activation of sterile inflammatory pathways and finally leads to chronic liver injury and fibrosis [10–12]. Of note, metabolic inflammation is primarily regulated by innate immune signaling. The innate immune system can sense metabolic stress, inducing inflammation and further worsening metabolic disorders, acting as a driving force in NAFLD progression [13–15]. Thus, disturbances of lipid metabolism and subsequent metabolic-stress-induced innate immune responses are believed to be pivotal in NAFLD pathogenesis (Figure 1).

Major Mechanisms in the Regulation of Hepatic Lipid Accumulation

Lipid metabolic disorder is the most direct etiology of NAFLD. When free fatty acids (FFAs) are either oversupplied or their disposal is damaged, they may be used as substrates for the production of lipotoxic species (e.g. ceramides, diacylglycerols, and lysophosphatidyl choline species) that trigger inflammation and fibrogenesis [2,10]. Therefore, clarifying the origins and fates of energy substrates in hepatocytes, especially FFAs, is essential for understanding the pathogenic underpinnings of

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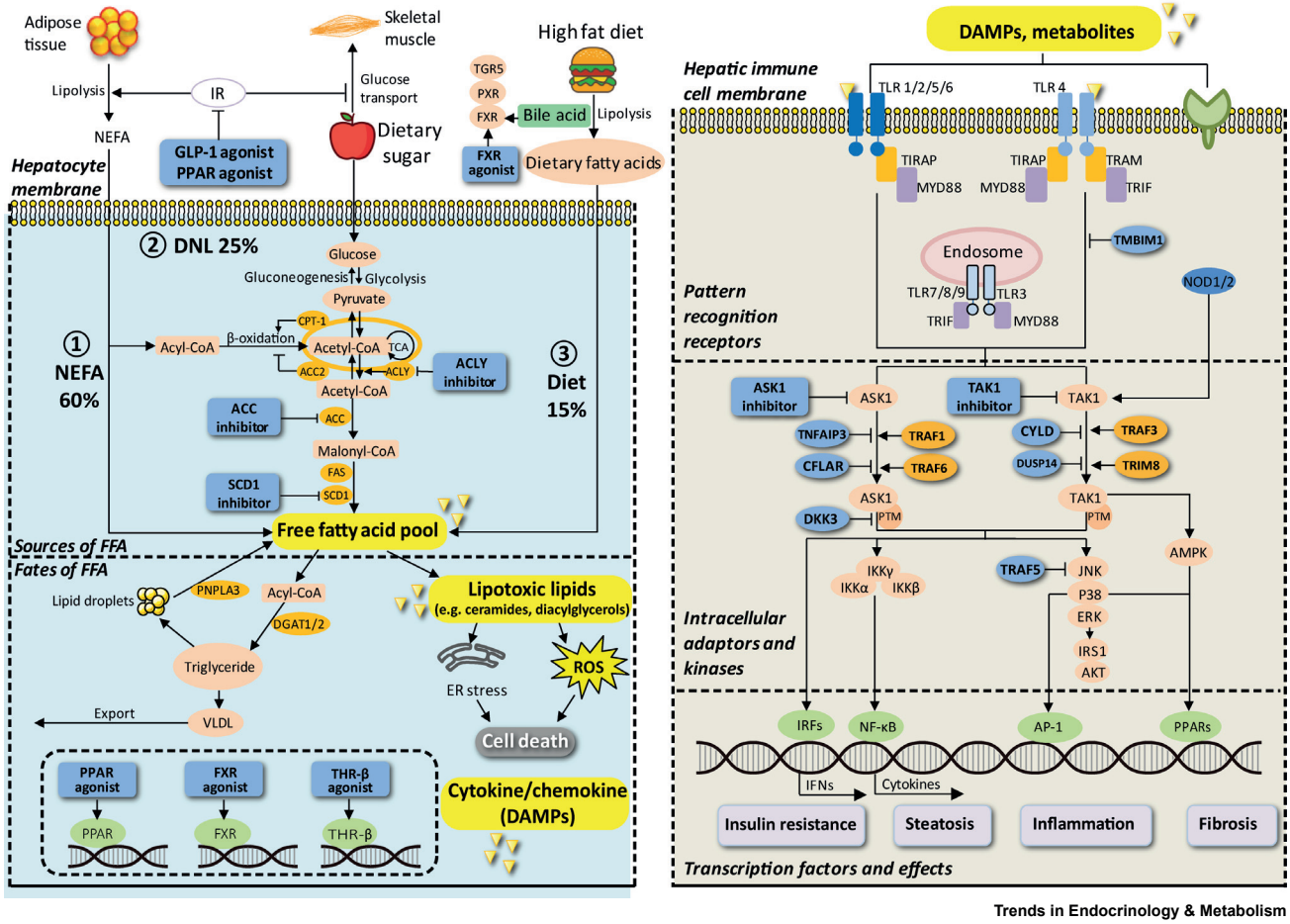

Figure 1. Lipid Metabolism and Metabolic-Stress-Induced Innate Immune Signaling in Nonalcoholic Fatty Liver Disease (NAFLD).
There are three sources of free fatty acids (FFAs) for the accumulation of lipid in the liver: 60% from the peripheral lipolysis or none-esterified fatty acid (NEFA) pool, 25% from *de novo* lipogenesis (DNL), and the remaining 15% from the diet. The two major metabolic fates of FFAs are mitochondrial β-oxidation and esterification to form triglyceride (TG). TGs can be exported into the blood as very-low-density lipoprotein (VLDL) and an excess of TGs are stored in lipid droplets. When FFAs are oversupplied or their disposal is damaged, excessive FFAs serve as substrates to generate lipotoxic species, which provoke endoplasmic reticulum (ER) stress, produce reactive oxygen species (ROS), and lead to cell death and release of danger-associated molecular patterns (DAMPs). Pattern recognition receptors (PRRs) such as TLRs and NLRs sense the continuously produced DAMPs and metabolites, thus triggering downstream signaling pathways. Apoptosis signal-regulating kinase 1 (ASK1) and TGF-β-activated kinase 1 (TAK1) are pivotal intracellular signaling transduction components, which are activated through post-transcriptional modification (PTM) and further activate several crucial kinases such as C-Jun N-terminal kinase (JNK), AMP-activated kinase (AMPK), and IκB. The activation of transcription factors such as interferon regulatory factors (IRFs), nuclear factor (NF)-κB, activator protein 1 (AP-1), and peroxisome proliferator-activated receptors (PPARs), is a common endpoint of these intracellular signaling cascades, which leads to the production of inflammatory cytokines and chemokines. These effector molecules can induce insulin resistance (IR), aggravate steatosis and inflammation, and promote fibrogenesis. Several endogenous targets that regulate altered innate immune elements in nonalcoholic steatohepatitis have been identified recently, including CASP8 and FADD-like apoptosis regulator (CFLAR), tumor necrosis factor (TNF) α-induced protein 3 (TNFAIP3), cylindromatosis (CYLD), transmembrane BAX inhibitor motif-containing 1 (TMBIM1), TNF receptor-associated factor 6 (TRAF6), TRAF1, TRAF3, dual-specificity phosphatase 14 (DUSP14), tripartite motif 8 (TRIM8), dickkopf-3 (DKK3), and TRAF5. Emerging therapeutic agents for NAFLD and their molecular targets are also shown.

NAFLD. Three sources of FFAs are known to contribute to lipid accumulation in the liver: 60% from the peripheral lipolysis or nonesterified fatty acid (NEFA) pool, 25% from *de novo* lipogenesis (DNL), and the remaining 15% from the diet [8]. Herein, major mechanisms and key molecules in the regulation of hepatic lipid accumulation during NAFLD are summarized.

NEFAs

Over half of FFAs in the liver come from the NEFA pool. FFAs originating from the lipolysis of triglyceride (TG) in adipose tissue are primarily delivered to the liver through blood. Notably, lipolysis of TG is regulated by the actions of insulin on adipocytes [15]. Insulin resistance (IR), characterized by low insulin sensitivity and low glucose disposal in peripheral tissues, is an integral component of NAFLD pathogenesis. IR leads to uninhibited lipolysis in adipose tissue, resulting in an excessive influx of NEFA to the liver, which is further converted into fatty acyl-CoA in hepatocytes [16–18]. High plasma levels of glucagon and epinephrine (released into the blood during starvation and exercise) promote the transfer of cytoplasmic fatty acyl-CoA into the mitochondria with the help of carnitine palmitoyltransferase (CPT)-1. Once the fatty acyl-CoA is inside the mitochondrial matrix, it is broken down through β-oxidation to form acetyl-CoA, which enters the citric acid cycle and eventually forms several equivalents of ATP. By contrast, insulin (e.g. after meals) accelerates the esterification of FAs to form TG, and an excess of TG is stored in the lipid droplets of hepatocytes [19].

DNL

The second major source of FFAs in hepatocytes is DNL, which is markedly enhanced in NAFLD patients [20]. Through DNL, hepatocytes convert excess glucose and fructose to FAs. Carbohydrate absorbed from the diet is transported to its target tissues by blood, which is mediated by the effects of insulin. Skeletal muscle IR hampers insulin-stimulated glucose transport into the myocytes, thus redirecting ingested glucose to the liver [10,19]. The increased level of glucose in hepatocytes is metabolized via glycolysis to generate pyruvic acid, which is then transformed to acetyl-CoA. Acetyl-CoA can be utilized in the Krebs cycles to generate ATP and promote gluconeogenesis during hypoglycemia, while in the case of hyperglycemia or IR, it is primarily processed via the DNL process to synthesize FAs [15,21]. The first committed step of DNL is the synthesis of malonyl-CoA from cytosolic acetyl-CoA under the catalytic actions of acetyl-CoA carboxylase (ACC). Malonyl-CoA is then used as a substrate to form saturated FA, a process that is catalyzed by FA synthase (FAS). Stearoyl CoA desaturase (SCD)1 is an endoplasmic reticulum (ER) enzyme that is responsible for the formation of monounsaturated FAs from saturated FAs. When excessive FAs are synthesized, they will be converted to fatty acyl-CoA, which is further esterified into TGs and stored in hepatocytes [19].

Diet

Approximately 15% of FFAs in the liver originate from the diet. Bile acids excreted by hepatocytes aid digestion and absorption of TGs by hydrolyzing them to form nascent chylomicrons in the intestinal lumen, which is the main site for lipid absorption. The FAs from the hydrolysis of TGs are then taken up by adipose tissue and liver [22]. In addition to their functions as detergents, bile acids can regulate lipid and glucose metabolism in both hepatic and extrahepatic tissues through modulation of specific bile-acid-activated receptors, including farnesoid X receptor (FXR), pregnane X receptor (PXR), and Takeda G protein-coupled receptor (TGR)5 [22,23].

Lipid Export

The disposal of FFAs is another key link in determining FFA homeostasis. Once homeostasis is impaired, excessive FFAs can serve as substrates to generate lipotoxic species, which provoke ER stress, produce reactive oxygen species (ROS), and lead to cell death and release of cytokines and chemokines [4,10]. The two major metabolic fates of FFAs in hepatocytes are mitochondrial β-oxidation and esterification to form TG, the latter of which is generally considered an adaptive and protective response to lipid overload. The key enzymes regulating the synthesis of TG include diglyceride acyltransferase (DGAT)1 and DGAT2. TGs can be exported into the blood as very-low-density lipoprotein (VLDL), but this removal cannot compensate for the increased synthesis of TG in NAFLD patients. An excess of TG is stored in the lipid droplets in hepatocytes, which can also be hydrolyzed under the actions of hydrolases such as PNPLA3 to contribute to the FA pool [8,15].

Metabolic Stress-Induced Innate Immune Response and Inflammation in NAFLD

Metabolic-stress-induced inflammation is mainly driven by the innate immune system. Innate immune cells residing in the liver include Kupffer cells, dendritic cells, and lymphocytes. Additionally, hepatocytes and liver sinusoidal endothelial cells can function as immune cells when they are stressed [13,24,25]. In metabolic disorders, pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs), in these cells sense the continuously produced danger-associated molecular patterns (DAMPs) and metabolites, thereby triggering the downstream cascades of immune reaction signaling, which is implicated with several pivotal intracellular adaptors and kinases, such as apoptosis signal-regulating kinase (ASK)1 and TGF-β-activated kinase (TAK)1 [26–29]. The activation of transcription factors, such as interferon regulatory factors (IRFs) and nuclear factor (NF)-κB, is a common endpoint of these intracellular signaling pathways and leads to the production of effector molecules (e.g. inflammatory cytokines and chemokines) [30–32]. These effector molecules can aggravate chronic inflammation, activate hepatic stellate cells (HSCs) to promote collagen deposition, and impair post-receptor insulin signaling to induce IR [33–37]. Together, these results show that the innate immune system directly modulates the pathogenesis of NAFLD at multiple levels, acting as a driving force in NAFLD progression.

Emerging Targets in Lipid Metabolism and Innate Immunity in NASH

Although this review focuses on molecular targets in pharmacological intervention, it is worth emphasizing that lifestyle changes with diet and exercise remain the mainstay of NAFLD treatment. The American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology guidelines advise a weight loss of at least 3–5% to improve steatosis, but a greater weight loss (7–10%) is recommended to improve other histological features of NASH, including fibrosis [1]. A combination of a hypocaloric diet and moderate-intensity exercise appears to be the best strategy to sustain weight loss over time. Exercise alone may reduce hepatic steatosis in NAFLD patients, but its ability to improve inflammation and fibrosis remains uncertain [1]. The most major problem with lifestyle interventions is that they are hard to sustain for a long time. Indeed, one prospective trial with paired liver biopsies in 261 NASH patients showed that only half of the participants succeeded to achieve at least a 7% weight loss after 12 months [38]. Under such circumstances, the development of drugs for NASH is flourishing and advancing rapidly. A list of agents targeting key regulators in each specific stage during the development and progression of NASH has been designed and tested in clinical trials (Figure 2). Based on the mechanistic view of the pathogenesis of NAFLD/NASH described above, the following section summarizes emerging molecular targets in the regulation of lipid metabolism and innate immunity and the advances of their corresponding compounds in preclinical models and clinical trials (Figure 1 and Table 1).

Targets in Regulating NEFA-Generated Lipids

Glucagon-like Peptide (GLP)-1

GLP-1 is a peptide hormone generated through the proteolytic processing of proglucagon and secreted by L cells in the intestine [39]. It decreases postprandial glucagon production and enhances insulin secretion, thus improving IR and IR-facilitated TG lipolysis, reducing NEFA-originated FFA overload in the liver. GLP-1 also has central actions, including decreasing appetite and delaying gastric emptying [39,40]. Given the central role of IR in NAFLD pathogenesis and the strong link between diabetes and NAFLD, GLP-1 receptor agonists, a group of antidiabetic agents, have logically become a feasible therapeutic option for NASH. Animal studies demonstrated that GLP-1 receptor agonists reduced liver steatosis and inflammation and exerted antifibrotic effects by improving the HSC phenotype [41,42]. Liraglutide, a GLP-1 receptor agonist requiring daily injection, has been shown to be effective for the resolution of NASH in a pilot Phase II clinical trial (LEAN trial) [43]. Notably, considerable weight loss (mean 5.3 kg) was achieved in patients treated with liraglutide. Therefore, weight loss may be a primary mechanism involved in the beneficial effects of liraglutide. However, some adverse events, such as gastrointestinal reactions, were observed. Thus, trials with a large sample size are necessary in the future. Semaglutide, another GLP-1 receptor agonist that is administered only once weekly, is currently being tested in a Phase II clinical trial.

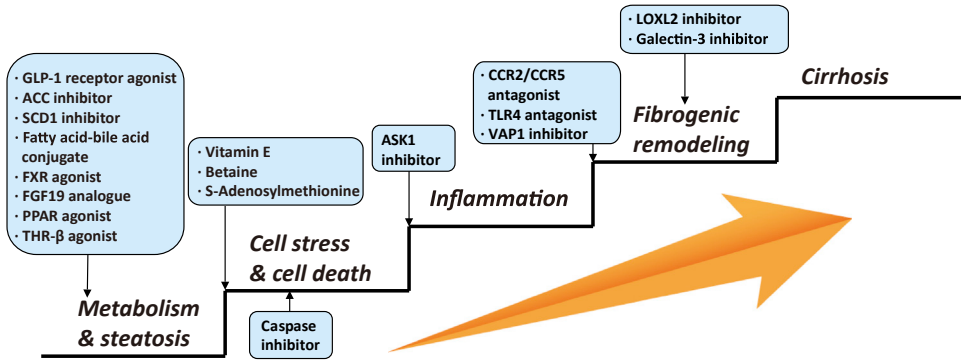

Figure 2. Current Therapeutic Targets for Nonalcoholic Steatohepatitis (NASH).
Metabolic-disorder-induced steatosis is likely the first step in the pathogenesis of NASH. A partial list of agents targeting key regulators in hepatic metabolism are shown, including glucagon-like peptide-1 (GLP-1) receptor agonist, acetyl-CoA carboxylase (ACC) inhibitor, stearoyl CoA desaturase 1 (SCD1) inhibitor, fatty acid–bile acid conjugate, farnesoid X receptor (FXR) agonist, fibroblast growth factor 19 (FGF19) analog, peroxisome proliferator-activated receptor (PPAR) agonist, and thyroid hormone receptor β (THR-β) agonist. Excessive accumulation of lipids induces distinct cellular metabolic stress and subsequently causes lipotoxicity, which triggers cell death. Representative agents targeting this process include vitamin E, betaine, S-adenosylmethionine, and caspase inhibitor. Continuous metabolic stress and subsequent cell death activate inflammatory signaling. Apotosis signal-regulating kinase 1 (ASK1) inhibitor, CC-chemokine receptor 2/CC-chemokine receptor 5 (CCR2/CCR5) antagonist, Toll-like receptor 4 (TLR4) antagonist, and vascular adhesion protein 1 (VAP1) inhibitor are agents acting to alleviate inflammation. Finally, chronic inflammation induces fibrogenesis and drives the progression of fibrogenic remodeling, ultimately leading to cirrhosis over time. Representative agents that work at this stage include Lysyl oxidase like 2 (LOXL2) inhibitor and galectin-3 inhibitor.

Targets in Regulating DNL-Generated Lipids

ACC

ACC is a key enzyme in the DNL process that catalyzes the starting step, the production of malonyl-CoA from acetyl-CoA [44]. There are two isoforms of ACC: the cytoplasmic isoform ACC1 and the mitochondrial isoform ACC2. Malonyl-CoA produced by ACC2 suppresses the mitochondrial FFA transfer protein CPT-1, leading to the inhibition of β-oxidation of fatty acyl-CoA [45,46]. Thus, inhibition of ACC appears to be a viable strategy to treat NASH as it suppresses DNL and promotes mitochondrial FFA β-oxidation. Animal studies using ACC knockout mice or ACC inhibitors showed inconsistent results among studies, as some reported decreased liver TG and improved hepatic insulin action, whereas others did not find these improvements [47–50]. A Phase II clinical trial showed that treatment with GS-0976 (which is a potent ACC inhibitor) at a higher dose for 12 weeks significantly reduced hepatic steatosis and fibrosis marker TIM1 in patients with biopsy-proven NASH and F1–F3 fibrosis [51]. A limitation of GS-0976 is the increase in serum TG levels, possibly due to a compensatory increase in sterol regulatory element-binding protein 1 activity, which leads to TG accumulation from peripheral FFAs [44]. Data from long-term clinical trials are urgently needed.

ATP-Citrate Lyase (ACLY)

ACLY is a lipogenic enzyme that catalyzes the cleavage of citrate to generate oxaloacetate and acetyl-CoA. It serves as a metabolic checkpoint for sensing excess nutrients and is positioned at the nexus of glucose catabolism and lipid synthesis [52,53]. Several studies have shown that the liver samples of NAFLD patients have increased mRNA, protein, and protein acetylation levels of ACLY, which may promote NAFLD progression [54,55]. Moreover, liver-specific ACL abrogation using adenovirus-mediated RNAi prevents liver steatosis and ameliorates hyperglycemia in leptin-receptor-deficient mice [53]. A recent mendelian randomization study, which uses genetic variants to mimic the effect

Table 1. Emerging Molecular Targets and Associated Agents with Clinical Data in NAFLD/NASH

Mechanisms	Agents	Status	Clinical data	NCT number
Targeting lipid generated from NEFA				
GLP-1 receptor agonist	Liraglutide	Phase II completed; Phase III recruiting	NASH resolution; improvements in fibrosis; weight loss; gastrointestinal reactions	01237119 02654665
	Semaglutide	Phase II active	Data pending	02970942
Targeting lipid generated from DNL				
ACC inhibitor	GS-0976	Phase II completed	Reduced steatosis and fibrosis marker; increased serum triglyceride	02856555
	PF-05221304	Phase II completed	DNL inhibition without triglyceride increase	03448172 03248882
SCD1 inhibitor	Armachol	Phase II completed	Improvements in hepatic steatosis; no alterations in liver enzymes and glucose metabolism	01094158 02279524
Targeting lipid generated from diet				
FXR agonist	Obeticholic acid	Phase II completed, Phase III recruiting	Improvements in NAS and fibrosis; no alterations in NASH resolution; pruritus and dyslipidemia	01265498 02548351 03439254
	GS-9674	One Phase II completed, another active	Reduced steatosis at higher dose	02854605 03449446
	LJN-452	Phase II recruiting	Interim results showed reduced ALT and liver fat content	02855164
Targeting multiple metabolic pathways				
PPARγ agonist	Pioglitazone	Phase III completed	NASH resolution; improvements in steatosis, NAS, and fibrosis; fluid retention, weight gain, and bone loss	00063622 00994682
PPARα/δ agonist	Elafibranor	Phase II completed, Phase III recruiting	Reduced fibrosis; improved metabolic parameters; failed to achieve NASH resolution without fibrosis worsening	01694849 02704403
PPARα/γ agonist	Saroglitazar	Phase II active	Data pending	03061721
THR-β agonist	MGL-3196	Phase II active	Reduced liver fat content and ALT	02912260
	VK2809	Phase II completed	Reduced liver fat content	02927184
Targeting Innate immune signaling				
ASK1 inhibitor	Selonsertib	Phase III completed	Failed to achieve ≥ 1-stage histologic improvement in fibrosis without worsening of NASH	02466516 03053050 03053063
TAK1 inhibitor	NA	Preclinical	NA	NA

of ACLY inhibitors and statins, showed that genetic inhibitions of ACLY and 3-hydroxy-3-methylglutaryl-CoA reductase led to similar patterns of changes in plasma lipid levels, and were associated with nearly identical effects on CVD risk per unit decrease in the LDL–cholesterol level [56]. These clues indicate that ACLY serves as a novel therapeutic target for modulating lipid metabolism and FLD. Excitingly, the crystal structure of ACLY has been reported recently, which provides the possibility for the development of molecular targeted therapeutic drugs in the future [52].

SCD1

SCD1 is an ER enzyme that catalyzes the synthesis of monounsaturated FAs from saturated FAs, which is the rate-limiting step in hepatocyte lipogenesis [57]. Liver-specific SCD1 knockout mice are protected from high-carbohydrate-diet-induced hepatic lipid accumulation [58]. In addition, synthetic antisense oligonucleotides (ASOs) targeting SCD1 protect mice against NAFLD and increase energy expenditure [59]. Aramchol, an FA–cholic acid conjugate, is able to inhibit SCD-1 and DNL, reduce steatosis and inflammation, and improve fibrosis in preclinical animal models [60]. A Phase II clinical trial showed that aramchol treatment for 3 months significantly reduced hepatic lipid content by 12.6% in patients with simple steatosis. However, no improvements in liver enzymes, glucose metabolism, and insulin sensitivity were observed [61]. Therefore, aramchol appears to be effective in alleviating liver steatosis, but its effects on inflammation and fibrosis need further investigation. An international multicenter Phase III trial evaluating the efficacy of aramchol in patients with biopsy-confirmed noncirrhotic NASH is ongoing.

Targets in Regulating Dietary-Generated Lipids

FXR

FXR is a bile acid nuclear receptor that is highly expressed in the liver and small intestinal mucosa. It has recently been recognized as a master regulator in glucose and lipid metabolism. After binding to bile acids, FXR is activated and induces gene transcription, resulting in various effects, including inhibition of lipogenesis and gluconeogenesis, restitution of insulin sensitivity, and suppression of bile acid synthesis [62]. FXR also stimulates the local production of fibroblast growth factor (FGF)15 (mice) or FGF19 (humans) in the intestine, which promotes mitochondrial FFA oxidation and hepatic glycogen synthesis after binding to FGF receptor (FGFR)4 in hepatocytes [63]. Mice with FXR deletion exhibit increased susceptibility of hepatic steatosis, necrotic inflammation, and fibrosis [64,65]. In NASH patients, the expression levels of FXR in the liver is downregulated and inversely correlated with disease severity [66]. Therefore, FXR agonists are reasonable pharmaceuticals to treat NASH. Obeticholic acid (OCA) is a semisynthetic FXR agonist with an approximately 100-fold higher potency than that of chenodeoxycholic acid [22]. It has been shown to be effective in improving glucose homeostasis and IR in obese and diabetic mice [67]. In the Phase II FLINT trial, patients treated with OCA showed significantly improved NAFLD activity score (NAS) and mean fibrosis stage compared to patients treated with placebo. However, the resolution of NASH and advanced fibrosis did not significantly differ between the groups. Moreover, some daunting side effects, such as pruritus and dyslipidemia, have been shown to be associated with the use of OCA [23]. To investigate these concerns, two Phase III trials (REGENERATE and REVERSE) are underway. In addition to OCA, several non-bile acid FXR agonists, such as LJN452 and GS9674, are currently being investigated in clinical trials.

Targets Regulating Multiple Metabolic Pathways

Peroxisome Proliferator-Activated Receptors (PPARs)

PPARs form a nuclear receptor superfamily that is deeply involved in the regulation of glucose and lipid metabolism as well as inflammatory pathways [68]. There are three isotypes of PPARs: PPARα, PPARδ, and PPARγ. PPARγ is mainly expressed in adipocytes and pancreatic β cells and is expressed at lower levels in hepatocytes and skeletal myocytes. It accelerates adipocyte differentiation, improves pancreatic β-cell function, and enhances insulin sensitivity and FFA oxidation in hepatocytes and muscle cells [68,69]. Thiazolidinediones (e.g. pioglitazone), a type of PPARγ agonist, have been studied in several trials and have been shown to be effective in improving histological manifestations of NASH, especially in patients with diabetes [70–73]. An important randomized placebo-controlled trial which included 101 patients with prediabetes or T2DM and biopsy-proven NASH followed for 3 years showed that 58% of pioglitazone-treated patients achieved the primary outcome of a reduction of at least 2 points in the NAS in two histological categories without worsening of fibrosis (treatment difference, 41 percentage points) [70]. In addition, pioglitazone treatment was associated with a higher proportion of NASH resolution and improvement in individual histologic scores such as the fibrosis score, compared with the placebo. In contrast to studies assessing the effects of pioglitazone in diabetic NASH patients, the Pioglitazone vs Vitamin E vs Placebo for the Treatment of Nondiabetic Patients with NASH (PIVENS) trial, which randomized 247 nondiabetic NASH patients to receive

vitamin E, pioglitazone, or placebo for 96 weeks, showed that pioglitazone treatment failed to achieve the primary outcome (as assessed by a composite of standardized scores for histological features) but significantly improved steatosis, lobular inflammation, and NAS [74]. However, the benefits of pioglitazone should be weighed against its side effects, such as fluid retention, weight gain, and osteoporotic fracture.

PPARα is most prominently expressed in the liver. It promotes hepatic FFA oxidation and protects hepatocytes from oxidative stress [75,76]. PPARδ is expressed more widely than PPARα. It inhibits lipogenesis, enhances FFA oxidation, and exerts anti-inflammatory effects [68]. Elafibranor (GFT505) is a typical PPARα/δ dual agonist, which revealed evident hepatoprotective effects in murine models of NASH [77]. The Phase IIb Elafibranor - (GFT505) NASH Treatment - GOLDEN 505 Study demonstrated that treatment with elafibranor for 52 weeks failed to achieve the a priori endpoint as defined by NASH resolution without a worsening of fibrosis. However, post hoc analysis showed an improvement in both disease activity and fibrosis in patients with NASH and an NAS>4 who received elafibranor [78]. Currently, elafibranor is being tested in a pivotal Phase III trial (RESOLVE-IT).

Thyroid Hormone Receptor (THR)-β

THR-β is a type of thyroxine receptor predominantly expressed in the liver and particularly enhances hepatic cholesterol metabolism and fat burning. A THR-β-specific agonist is supposed to reduce liver steatosis while avoiding the adverse effects caused by THR-α stimulation of other organs, such as the cardiovascular system and bone [79]. MGL-3196 is an outstanding THR-β agonist that could reduce liver cholesterol and TG in diet-induced NAFLD mice [80]. A Phase IIa proof-of-concept trial with 125 NASH patients showed that MGL-3196 treatment for 12 weeks decreased the amount of liver fat by approximately 40%, with a low incidence of adverse reactions [81]. Therefore, the results of the Phase III trial of MGL-3196 are highly expected. VK2809 is another selective THR-β agonist, which remarkably increased hepatic FFA oxidation and reduced liver fat accumulation in preclinical rodent models [82]. Data from a Phase II clinical trial that evaluated the efficacy and safety of VK2809 in patients with NAFLD and hyperlipidemia are waiting to be published.

Targets Regulating Innate Immune Responses

ASK1

ASK1 is a member of the mitogen-activate protein kinase kinase kinase (MAP3K) family that activates downstream C-Jun N-terminal kinase (JNK)–p38 and NF-κB pathways. Under metabolic stress, ASK1 can recruit multiple regulatory factors due to its scaffold structure and then be activated through post-transcriptional modification (PTM). Activated ASK1 induces downstream signaling transduction and further induces inflammation, apoptosis, and fibrosis, which functions as a molecular hub regulating cellular signaling flow in NASH [83–88]. ASK1 deficiency significantly alleviated hepatic lipid accumulation in mice fed a high-fat diet [89]. In addition, ASK1 is hyperactivated in the liver of patients with NASH [9,11]. Thus, ASK1 is one of the prime targets for the development of drugs for NASH. A Phase II clinical trial showed that selonsertib (GS-4997), a selective inhibitor of ASK1, had a better antifibrotic effect than the control agent in NASH patients with fibrosis stage 2 or 3 [90]. The STELLAR-3 trial and the STELLAR-4 trial are two pivotal Phase III trials that recruited participants with NASH-induced bridging fibrosis (F3) and NASH-induced compensated cirrhosis, respectively. Unfortunately, selonsertib failed to achieve the primary endpoint (i.e. ≥ 1-stage histological improvement in fibrosis without worsening of NASH) at 48 weeks in both trials, as announced recently (https://www.gilead.com). In contrast to selonsertib, CASP8 and FADD-like apoptosis regulator (CFLAR) and TNF-α-induced protein (TNFAIP)3 inhibit ASK1 activation by damping its PTM process, which serve as key endogenous suppressors of ASK1 [91,92]. CFLAR and TNFAIP3 have been shown to ameliorate NASH in preclinical models and are promising therapeutic targets in future research.

TAK1

TAK1 is also a member of the MAP3K family, which serves as a pivotal regulator in innate immune signaling transduction. Metabolic stress can hyperactivate TAK1 by inducing its PTM, namely, ubiquitination and subsequent autophosphorylation, causing the activation of downstream JNK–p38 and

Outstanding Questions.

What are the central metabolic events that lead to the transition from simple steatosis to NASH? How can the most crucial molecules in the progression of NAFLD be determined?

Is targeting a single target sufficient to treat NASH? If not, what is the principle for choosing combined targets?

What are the tissue- and cell-specific functions of these molecular targets in the pathogenesis of NASH? How to antagonize their pathogenic effects while maintaining normal biological functions? Is it possible to design target-specific effectors contraposing cells with disordered metabolism or injured hepatic cells?

How to identify patients with a higher risk of advanced complications? How to optimize drug regimens based on risk stratification?

Is NAFLD a critical independent risk factor that can cause systemic metabolic diseases? In turn, what is the effect of systemic metabolic status on the metabolic and immunologic profiles of hepatic cells? Can medications used for diabetes influence the progression of NAFLD via inflammatory pathways other than metabolic homeostasis?

NF-κB signaling pathways, which further promote metabolic disorders and steatohepatitis [93–97]. Moreover, TAK1 weakens insulin signaling transduction through IRS1 phosphorylation and enhances PPAR expression via AMP-activated protein kinase (AMPK), thereby playing a crucial role in hepatic metabolic homeostasis [15,98]. Although excessive activation of TAK1 is harmful, maintaining its normal catalytic function in the regulation of lipid metabolism, oxidation, and cell autophagy is important. In fact, total deletion of TAK1 also promotes hepatosteatosis and inflammation in mice [99,100]. Thus, inhibiting TAK1 overactivation via deubiquitination or dephosphorylation without impairing its physiological activity could be an applicable strategy for treating NASH. Cylindromatosis (CYLD), which inhibits TAK1 activation through its deubiquitinase function, was found to be effective in suppressing NASH progression and is now a promising therapeutic target [95]. However, successful clinical translation of TAK1 inhibitors requires careful clinical trials in the future.

Concluding Remarks and Future Perspectives

NAFLD has become the most common chronic liver disease worldwide, and its presence and severity are strongly associated with increasing risks of end-stage liver disease, HCC, and serious extrahepatic diseases like CVD. Hence, effective treatments for NAFLD are eagerly awaited. A series of therapeutic targets have been identified, and some of their corresponding compounds have shown promise in early phase trials. Successful clinical translation requires an in-depth knowledge of the molecular mechanisms of the disease (see Outstanding Questions). Disequilibrium of lipid metabolism and metabolic-stress-induced inflammation are believed to be central in NAFLD pathogenesis. Of note, metabolic inflammation is primarily mediated by innate immune signaling, which is increasingly recognized as an integral role in the progression of NAFLD. However, as NAFLD is a complex disease involving a myriad of genetic, metabolic, and environmental factors, we still lack a comprehensive understanding of the mechanistic differences in various stages of NAFLD and the forces driving the progression from one stage to the next. To overcome the intractable challenge of the considerable heterogeneity in NASH pathogenesis, recent advances in systems-based multiomic analyses, combined with big data technology, will be useful for elucidating mechanisms, screening potential therapeutic targets, and providing individualized therapies in the future. Furthermore, methodologies in artificial intelligence, such as machine learning, are increasingly being applied to the interpretation of omics data as they enable integrative analyses among high-dimensional data sets. In addition, the development of more clinically relevant animal models, especially nonhuman primate models, will facilitate translation from the laboratory to the clinic. Since NAFLD is a multifactorial disorder, combination therapies engaging different targets may be necessary. Future clinical trials with adequate duration and power are needed to evaluate the long-term efficacy and safety of each potential therapeutic option.

Disclaimer Statement

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References

1. Chalasani, N., *et al.* (2018) The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 67, 328–357
2. Schuster, S., *et al.* (2018) Triggering and resolution of inflammation in NASH. *Nat. Rev. Gastroenterol. Hepatol.* 15, 349–364
3. Lonardo, A., *et al.* (2018) Hypertension, diabetes, atherosclerosis and NASH: cause or consequence? *J. Hepatol.* 68, 335–352
4. Sanyal, A.J. (2019) Past, present and future perspectives in nonalcoholic fatty liver disease. *Nat. Rev. Gastroenterol. Hepatol.* 16, 377–386
5. Zhou, F., *et al.* (2019) Unexpected rapid increase in the burden of nonalcoholic fatty liver disease in China from 2008 to 2018: a systematic review and meta-analysis. *Hepatology*. Published online May 9, 2019. https://doi.org/10.1002/hep.30702.
6. Zhang, X.J., *et al.* (2018) Time to step-up the fight against NAFLD. *Hepatology* 67, 2068–2071

7. Cai, J., *et al.* (2019) Progress and challenges in the prevention and control of nonalcoholic fatty liver disease. *Med. Res. Rev.* 39, 328–348
8. Arab, J.P., *et al.* (2018) Recent insights into the pathogenesis of nonalcoholic fatty liver disease. *Annu. Rev. Pathol.* 13, 321–350
9. Yu, Y., *et al.* (2019) Insights into the epidemiology, pathogenesis, and therapeutics of nonalcoholic fatty liver diseases. *Adv. Sci. (Weinh)* 6, 1801585
10. Friedman, S.L., *et al.* (2018) Mechanisms of NAFLD development and therapeutic strategies. *Nat. Med.* 24, 908–922
11. Cai, J., *et al.* (2018) Role of innate immune signaling in non-alcoholic fatty liver disease. *Trends Endocrinol. Metab.* 29, 712–722
12. Wang, X.A., *et al.* (2013) CARD3 deficiency exacerbates diet-induced obesity, hepatosteatosis, and insulin resistance in male mice. *Endocrinology* 154, 685–697
13. Cai, J., *et al.* (2019) The role of innate immune cells in nonalcoholic steatohepatitis. *Hepatology* 70, 1026–1037
14. Xu, M., *et al.* (2019) Innate immune signaling and its role in metabolic and cardiovascular diseases. *Physiol. Rev.* 99, 893–948
15. Bai, L., and Li, H. (2019) Innate immune regulatory networks in hepatic lipid metabolism. *J. Mol. Med. (Berl)* 97, 593–604
16. Fuchs, C.D., *et al.* (2014) Role of metabolic lipases and lipolytic metabolites in the pathogenesis of NAFLD. *Trends Endocrinol. Metab.* 25, 576–585
17. Luo, P., *et al.* (2016) Hepatic oncostatin M receptor beta regulates obesity-induced steatosis and insulin resistance. *Am. J. Pathol.* 186, 1278–1292
18. Wang, X.A., *et al.* (2013) Interferon regulatory factor 7 deficiency prevents diet-induced obesity and insulin resistance. *Am. J. Physiol. Endocrinol. Metab.* 305, E485–E495
19. Samuel, V.T., and Shulman, G.I. (2018) Nonalcoholic fatty liver disease as a nexus of metabolic and hepatic diseases. *Cell Metab.* 27, 22–41
20. Lambert, J.E., *et al.* (2014) Increased *de novo* lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology* 146, 726–735
21. Sunny, N.E., *et al.* (2011) Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. *Cell Metab.* 14, 804–810
22. Arab, J.P., *et al.* (2017) Bile acids and nonalcoholic fatty liver disease: molecular insights and therapeutic perspectives. *Hepatology* 65, 350–362
23. Neuschwander-Tetri, B.A., *et al.* (2015) Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 385, 956–965
24. Cai, J., *et al.* (2019) Innate immune signaling in nonalcoholic fatty liver disease and cardiovascular diseases. *Annu. Rev. Pathol.* 14, 153–184
25. Wang, W., *et al.* (2017) The innate immune signaling in cancer and cardiometabolic diseases: friends or foes? *Cancer Lett.* 387, 46–60
26. Zhang, Y., *et al.* (2017) Reprogramming innate immune signaling in cardiometabolic disease. *Hypertension* 69, 747–760
27. Zhao, G.N., *et al.* (2017) Tmbim1 is a multivesicular body regulator that protects against non-alcoholic fatty liver disease in mice and monkeys by targeting the lysosomal degradation of Tlr4. *Nat. Med.* 23, 742–752
28. Wang, Y., *et al.* (2019) Hepatocyte TRAF6 aggravates hepatic inflammation and fibrosis by promoting Lys6-linked polyubiquitination of ASK1. *Hepatology*. Published online June 20, 2019. https://doi.org/10.1002/hep.30822.
29. Gao, L., *et al.* (2016) Tumor necrosis factor receptor-associated factor 5 (Traf5) acts as an essential negative regulator of hepatic steatosis. *J. Hepatol.* 65, 125–136
30. Zhang, Y., *et al.* (2017) Targeting interferon regulatory factor for cardiometabolic diseases: opportunities and challenges. *Curr. Drug Targets* 18, 1754–1778
31. Zhang, X.J., *et al.* (2015) Interferon regulatory factor signalings in cardiometabolic diseases. *Hypertension* 66, 222–247
32. Zhao, G.N., *et al.* (2015) Interferon regulatory factors: at the crossroads of immunity, metabolism, and disease. *Biochim. Biophys. Acta* 1852, 365–378
33. Zhang, Y., and Li, H. (2017) Reprogramming interferon regulatory factor signaling in cardiometabolic diseases. *Physiology (Bethesda)* 32, 210–223
34. Wang, P.X., *et al.* (2015) Interferon regulatory factor 9 is a key mediator of hepatic ischemia/reperfusion injury. *J. Hepatol.* 62, 111–120
35. Wang, X.A., *et al.* (2014) Interferon regulatory factor 3 constrains IKKbeta/NF-kappaB signaling to alleviate hepatic steatosis and insulin resistance. *Hepatology* 59, 870–885
36. Wang, X.A., *et al.* (2013) Interferon regulatory factor 9 protects against hepatic insulin resistance and steatosis in male mice. *Hepatology* 58, 603–616
37. Wang, P.X., *et al.* (2016) Liver capsule: IRFs in hepatocytes: pathophysiology. *Hepatology* 63, 1706
38. Vilar-Gomez, E., *et al.* (2015) Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. *Gastroenterology* 149, 367–378.e5, quiz e14-5.
39. Armstrong, M.J., *et al.* (2016) Glucagon-like peptide 1 decreases lipotoxicity in non-alcoholic steatohepatitis. *J. Hepatol.* 64, 399–408
40. Jinnouchi, H., *et al.* (2015) Liraglutide, a glucagon-like peptide-1 analog, increased insulin sensitivity assessed by hyperinsulinemic-euglycemic clamp examination in patients with uncontrolled type 2 diabetes mellitus. *J. Diabetes Res.* 2015, 706416
41. de Mesquita, F.C., *et al.* (2015) Deactivation of human and rat cirrhotic hepatic stellate cells: a novel alternative use for the glucagon-like 1 receptor agonist Liraglutide. *Hepatology* 62, 901A
42. Valdecantos, M.P., *et al.* (2017) A novel glucagon-like peptide 1/glucagon receptor dual agonist improves steatohepatitis and liver regeneration in mice. *Hepatology* 65, 950–968
43. Armstrong, M.J., *et al.* (2016) Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* 387, 679–690
44. Kim, C.W., *et al.* (2017) Acetyl CoA carboxylase inhibition reduces hepatic steatosis but elevates plasma triglycerides in mice and humans: a bedside to bench investigation. *Cell Metab.* 26, 576
45. Fullerton, M.D., *et al.* (2013) Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. *Nat. Med.* 19, 1649–1654
46. Choi, C.S., *et al.* (2007) Continuous fat oxidation in acetyl-CoA carboxylase 2 knockout mice increases total energy expenditure, reduces fat mass, and improves insulin sensitivity. *Proc. Natl. Acad. Sci. U. S. A.* 104, 16480–16485
47. Goedeke, L., *et al.* (2018) Acetyl-CoA carboxylase inhibition reverses NAFLD and hepatic insulin resistance but promotes hypertriglyceridemia in rodents. *Hepatology* 68, 2197–2211

48. Harriman, G., *et al.* (2016) Acetyl-CoA carboxylase inhibition by ND-630 reduces hepatic steatosis, improves insulin sensitivity, and modulates dyslipidemia in rats. *Proc. Natl. Acad. Sci. U. S. A.* 113, E1796–E1805
49. Chow, J.D., *et al.* (2014) Genetic inhibition of hepatic acetyl-CoA carboxylase activity increases liver fat and alters global protein acetylation. *Mol. Metab.* 3, 419–431
50. Olson, D.P., *et al.* (2010) Gene knockout of Acc2 has little effect on body weight, fat mass, or food intake. *Proc. Natl. Acad. Sci. U. S. A.* 107, 7598–7603
51. Loomba, R., *et al.* (2018) GS-0976 reduces hepatic steatosis and fibrosis markers in patients with nonalcoholic fatty liver disease. *Gastroenterology* 155, 1463–1473, e1466.
52. Verschueren, K.H.G., *et al.* (2019) Structure of ATP citrate lyase and the origin of citrate synthase in the Krebs cycle. *Nature* 568, 571–575
53. Wang, Q., *et al.* (2009) Abrogation of hepatic ATP-citrate lyase protects against fatty liver and ameliorates hyperglycemia in leptin receptor-deficient mice. *Hepatology* 49, 1166–1175
54. Guo, L., *et al.* (2019) Enhanced acetylation of ATP-citrate lyase promotes the progression of nonalcoholic fatty liver disease. *J. Biol. Chem.* 294, 11805–11816
55. Ahrens, M., *et al.* (2013) DNA methylation analysis in nonalcoholic fatty liver disease suggests distinct disease-specific and remodeling signatures after bariatric surgery. *Cell Metab.* 18, 296–302
56. Ference, B.A., *et al.* (2019) Mendelian randomization study of ACLY and cardiovascular disease. *N. Engl. J. Med.* 380, 1033–1042
57. Ajmera, V.H., *et al.* (2019) MRI assessment of treatment response in HIV-associated NAFLD: a randomized trial of a stearoyl-coenzyme-a-desaturase-1 inhibitor (ARRIVE trial). *Hepatology*. Published online April 23, 2019. https://doi.org/10.1002/hep.30674.
58. Miyazaki, M., *et al.* (2007) Hepatic stearoyl-CoA desaturase-1 deficiency protects mice from carbohydrate-induced adiposity and hepatic steatosis. *Cell Metab.* 6, 484–496
59. Jiang, G., *et al.* (2005) Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-CoA desaturase-1. *J. Clin. Invest.* 115, 1030–1038
60. Iruarrizaga-Lejarreta, M., *et al.* (2017) Role of aramchol in steatohepatitis and fibrosis in mice. *Hepatol. Commun.* 1, 911–927
61. Safadi, R., *et al.* (2014) The fatty acid-bile acid conjugate aramchol reduces liver fat content in patients with nonalcoholic fatty liver disease. *Clin. Gastroenterol. Hepatol.* 12, 2085–2091, e2081.
62. Anstee, Q.M., *et al.* (2019) From NASH to HCC: current concepts and future challenges. *Nat. Rev. Gastroenterol. Hepatol.* 16, 411–428
63. Jahn, D., *et al.* (2015) Mechanisms of enterohepatic fibroblast growth factor 15/19 signaling in health and disease. *Cytokine Growth Factor Rev.* 26, 625–635
64. Sinal, C.J., *et al.* (2000) Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell* 102, 731–744
65. Kong, B., *et al.* (2009) Farnesoid X receptor deficiency induces nonalcoholic steatohepatitis in low-density lipoprotein receptor-knockout mice fed a high-fat diet. *J. Pharmacol. Exp. Ther.* 328, 116–122
66. Min, H.K., *et al.* (2012) Increased hepatic synthesis and dysregulation of cholesterol metabolism is associated with the severity of nonalcoholic fatty liver disease. *Cell Metab.* 15, 665–674
67. Zhang, Y., *et al.* (2006) Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proc. Natl. Acad. Sci. U. S. A.* 103, 1006–1011
68. Gross, B., *et al.* (2017) PPARs in obesity-induced T2DM, dyslipidaemia and NAFLD. *Nat. Rev. Endocrinol.* 13, 36–49
69. Tong, J., *et al.* (2019) Hepatic interferon regulatory factor 6 alleviates liver steatosis and metabolic disorder by transcriptionally suppressing peroxisome proliferator-activated receptor gamma in mice. *Hepatology* 69, 2471–2488
70. Cusi, K., *et al.* (2016) Long-term pioglitazone treatment for patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus: a randomized trial. *Ann. Intern. Med.* 165, 305–315
71. Mahady, S.E., *et al.* (2011) The role of thiazolidinediones in non-alcoholic steatohepatitis - a systematic review and meta analysis. *J. Hepatol.* 55, 1383–1390
72. Bril, F., *et al.* (2018) Response to pioglitazone in patients with nonalcoholic steatohepatitis with vs without type 2 diabetes. *Clin. Gastroenterol. Hepatol.* 16, 558–566.e552.
73. Belfort, R., *et al.* (2006) A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N. Engl. J. Med.* 355, 2297–2307
74. Sanyal, A.J., *et al.* (2010) Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N. Engl. J. Med.* 362, 1675–1685
75. Zhu, L.H., *et al.* (2014) Mindin/Spondin 2 inhibits hepatic steatosis, insulin resistance, and obesity via interaction with peroxisome proliferator-activated receptor alpha in mice. *J. Hepatol.* 60, 1046–1054
76. Pawlak, M., *et al.* (2015) Molecular mechanism of PPARalpha action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. *J. Hepatol.* 62, 720–733
77. Staels, B., *et al.* (2013) Hepatoprotective effects of the dual peroxisome proliferator-activated receptor alpha/delta agonist, GFT505, in rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Hepatology* 58, 1941–1952
78. Ratziu, V., *et al.* (2016) Elafibranor, an agonist of the peroxisome proliferator-activated receptor-alpha and -delta, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. *Gastroenterology* 150, 1147–1159, e1145.
79. Sinha, R.A., *et al.* (2014) Thyroid hormone regulation of hepatic lipid and carbohydrate metabolism. *Trends Endocrinol. Metab.* 25, 538–545
80. Kelly, M.J., *et al.* (2014) Discovery of 2-[3,5-dichloro-4-(5-isopropyl-6-oxo-1,6-dihydropyridazin-3-yloxy) phenyl]-3,5-dio xo-2,3,4,5-tetrahydro[1,2,4]triazine-6-carbonitrile (MGL-3196), a highly selective thyroid hormone receptor beta agonist in clinical trials for the treatment of dyslipidemia. *J. Med. Chem.* 57, 3912–3923
81. Ogawa, Y., *et al.* (2019) Present and emerging pharmacotherapies for non-alcoholic steatohepatitis in adults. *Expert. Opin. Pharmacother.* 20, 69–82
82. Cable, E.E., *et al.* (2009) Reduction of hepatic steatosis in rats and mice after treatment with a liver-targeted thyroid hormone receptor agonist. *Hepatology* 49, 407–417
83. Bai, L., *et al.* (2019) F-box/WD repeat-containing protein 5 mediates the ubiquitination of apoptosis signal-regulating kinase 1 and exacerbates nonalcoholic steatohepatitis in mice. *Hepatology*. Published online January 31, 2019. https://doi.org/10.1002/hep.30537.
84. Xiang, M., *et al.* (2016) Targeting hepatic TRAF1-ASK1 signaling to improve inflammation, insulin resistance, and hepatic steatosis. *J. Hepatol.* 64, 1365–1377

85. Xie, L., *et al.* (2016) DKK3 expression in hepatocytes defines susceptibility to liver steatosis and obesity. *J. Hepatol.* 65, 113–124
86. Qin, J.J., *et al.* (2018) Caspase recruitment domain 6 protects against hepatic ischemia/reperfusion injury by suppressing ASK1. *J. Hepatol.* 69, 1110–1122
87. Chen, L., *et al.* (2017) Tripartite motif 8 contributes to pathological cardiac hypertrophy through enhancing transforming growth factor beta-activated kinase 1-dependent signaling pathways. *Hypertension* 69, 249–258
88. Yan, Z.Z., *et al.* (2019) Integrated omics reveals tollip as an regulator and therapeutic target for hepatic ischemia-reperfusion injury in mice. *Hepatology*. Published online May 11, 2019. https://doi.org/10.1002/hep.30705.
89. Yamamoto, E., *et al.* (2008) Olmesartan prevents cardiovascular injury and hepatic steatosis in obesity and diabetes, accompanied by apoptosis signal regulating kinase-1 inhibition. *Hypertension* 52, 573–580
90. Loomba, R., *et al.* (2017) The ASK1 inhibitor selonsertib in patients with nonalcoholic steatohepatitis: a randomized, phase 2 trial. *Hepatology* 67, 549–559
91. Wang, P.X., *et al.* (2017) Targeting CASP8 and FADD-like apoptosis regulator ameliorates nonalcoholic steatohepatitis in mice and nonhuman primates. *Nat. Med.* 23, 439–449
92. Zhang, P., *et al.* (2018) The deubiquitinating enzyme TNFAIP3 mediates inactivation of hepatic ASK1 and ameliorates nonalcoholic steatohepatitis. *Nat. Med.* 24, 84–94
93. Ji, Y.X., *et al.* (2016) The ubiquitin E3 ligase TRAF6 exacerbates pathological cardiac hypertrophy via TAK1-dependent signalling. *Nat. Commun.* 7, 11267
94. Wang, P.X., *et al.* (2016) Hepatocyte TRAF3 promotes liver steatosis and systemic insulin resistance through targeting TAK1-dependent signalling. *Nat. Commun.* 7, 10592
95. Ji, Y.X., *et al.* (2018) The deubiquitinating enzyme cylindromatosis mitigates nonalcoholic steatohepatitis. *Nat. Med.* 24, 213–223
96. Wang, X., *et al.* (2017) Dusp14 protects against hepatic ischaemia-reperfusion injury via Tak1 suppression. *J. Hepatol.* Published online September 6, 2017. https://doi.org/10.1016/j.jhep.2017.08.032.
97. Hu, J., *et al.* (2016) Targeting TRAF3 signaling protects against hepatic ischemia/reperfusions injury. *J. Hepatol.* 64, 146–159
98. Yan, F.J., *et al.* (2017) The E3 ligase tripartite motif 8 targets TAK1 to promote insulin resistance and steatohepatitis. *Hepatology* 65, 1492–1511
99. Inokuchi-Shimizu, S., *et al.* (2014) TAK1-mediated autophagy and fatty acid oxidation prevent hepatosteatosis and tumorigenesis. *J. Clin. Invest.* 124, 3566–3578
100. Morioka, S., *et al.* (2016) TAK1 regulates hepatic lipid homeostasis through SREBP. *Oncogene* 35, 3829–3838