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REVIEW

Metabolic Targets in Nonalcoholic Fatty Liver Disease

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SUMMARY

This review highlights the major metabolic drivers of nonalcoholic fatty liver disease pathogenesis and gives a current overview of the treatment landscape for nonalcoholic steatohepatitis.

The prevalence and diagnosis of nonalcoholic fatty liver disease (NAFLD) is on the rise worldwide and currently has no FDA-approved pharmacotherapy. The increase in disease burden of NAFLD and a more severe form of this progressive liver disease, nonalcoholic steatohepatitis (NASH), largely mirrors the increase in obesity and type 2 diabetes (T2D) and reflects the hepatic manifestation of an altered metabolic state. Indeed, metabolic syndrome, defined as a constellation of obesity, insulin resistance, hyperglycemia, dyslipidemia and hypertension, is the major risk factor predisposing the NAFLD and NASH. There are multiple potential pharmacologic strategies to rebalance aspects of disordered metabolism in NAFLD. These include therapies aimed at reducing hepatic steatosis by directly modulating lipid metabolism within the liver, inhibiting fructose metabolism, altering delivery of free fatty acids from the adipose to the liver by targeting insulin resistance and/or adipose metabolism, modulating glycemia, and altering pleiotropic metabolic pathways simultaneously. Emerging data from human genetics also supports a role for metabolic drivers in NAFLD and risk for progression to NASH. In this review, we highlight the prominent metabolic drivers of NAFLD pathogenesis and discuss the major metabolic targets of NASH pharmacotherapy. ***(Cell Mol Gastroenterol Hepatol 2019;8:247–267; https://doi.org/10.1016/j.jcmgh.2019.04.007)***

Nonalcoholic fatty liver disease (NAFLD) is a constellation of conditions that originates with excess accumulation of fat within the liver (defined as >5%). Nonalcoholic steatohepatitis (NASH) is a clinical and histological subset of NAFLD that is associated with increased all-cause mortality, cirrhosis and end-stage liver disease, increased cardiovascular mortality, and increased incidence of both liver-related and non–liver-related cancers.[1] NASH is diagnosed clinically by liver biopsy demonstrating steatosis, inflammation, and cytological ballooning of liver hepatocytes, often with varying degrees of fibrosis. NASH progresses with increasing degrees of fibrosis, with cirrhosis developing in a subset of patients,[1] with the most common complication of cirrhosis being hepatocellular carcinoma.[2]

Metabolic perturbations, including insulin resistance, impaired glycemic control, and altered lipid metabolism, have been hypothesized to contribute to the molecular pathogenesis of NAFLD and NASH (Figure 1). Steatosis is a necessary but not sufficient component of the pathogenesis of NASH.[3] Consistent with this, multiple studies have demonstrated that the severity of steatosis predicts the risk of concomitant steatohepatitis as well as the risk of progression to cirrhosis.[4–6] Genetic polymorphisms in humans, including the I148M polymorphism in the PNPLA3 gene, have also been shown to increase risk of steatosis and in turn increase risk for NASH.[7,8] These observations taken together provide molecular evidence that steatosis is a key pathogenic factor in NASH.

Hepatic steatosis is a consequence of an imbalance in triglyceride (TG) production or uptake into the liver and clearance or removal (Figure 1).[9] Elevated body mass index is a significant risk factor for steatosis[10] suggesting that excess caloric intake and obesity contribute to the development of NAFLD. Altering the balance of hepatic TG accumulation and removal by either (or both) reducing fat production or promoting fat clearance is likewise expected to reduce steatosis. Amelioration of steatosis in turn is hypothesized to reduce buildup of lipotoxic lipid species, suppress hepatic inflammation, and subsequently reduce fibrogenesis (Figure 1). Indeed, studies in animal models have demonstrated that multiple modalities which reduce steatosis result in downstream improvements in hepatic inflammation and fibrosis.[11–21]

Perhaps the most compelling data in support of targeting metabolic pathways in NASH comes from the bariatric

Abbreviations used in this paper: **ACC, acetyl-CoA carboxylase; ALT, alanine aminotransferase; ASO, anti-sense oligonucleotide; AST, aspartate aminotransferase; ChREBP, carbohydrate response element binding protein; CI, confidence interval; DGAT, diacylglycerol O-acyltransferase; DNL, de novo lipogenesis; FAS, fatty acid synthase; FFA, free fatty acid; FGF, fibroblast growth factor; FXR, Farnesoid X receptor; GLP-1, glucagon-like peptide-1; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; NAS, Nonalcoholic Fatty Liver Disease Activity Score; NASH, nonalcoholic steatohepatitis; OR, odds ratio; PDFF, proton density fat fraction; PPAR, peroxisome proliferator-activated receptor; SGLT2, sodium glucose co-transporter 2; SREBP-1c, sterol regulatory element binding protein-1c; T2D, type 2 diabetes; T2DM, type 2 diabetes mellitus; TG, triglyceride; TH, thyroid hormone; THR, thyroid hormone receptor; Treg, regulatory T cells; TZD, thiazolidinedione; VLDL, very low-density lipoprotein.**

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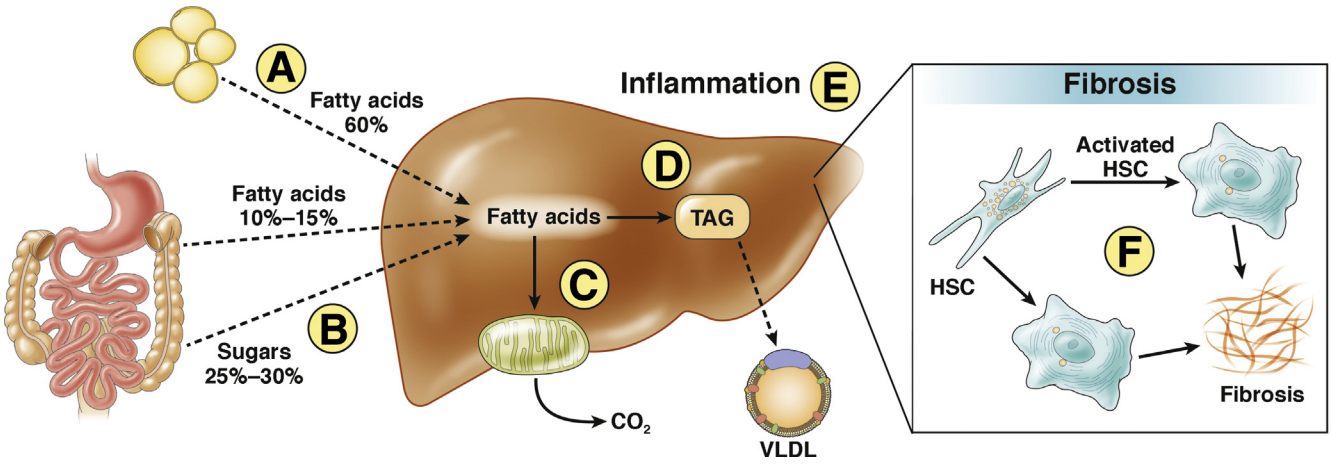

Figure 1. Model of hepatic lipid metabolism, inflammation, and fibrosis. Hepatic lipid content is a balance between uptake and synthesis of lipids and lipid disposal/export. The primary source of hepatic fat is the liberation of free fatty acids from adipose/fat tissue via lipolysis (*A*),[27,106,107] with additional uptake of fatty acids from the circulation (*B*) and de novo lipogenesis (*D*). Lipids are cleared from the liver by beta-oxidation of fatty acids in the mitochondria (*C*) and through export as very low-density lipoprotein (VLDL) particles. Excess hepatic lipid accumulation, on its own or in combination with additional environmental insults, can trigger an inflammatory response in the liver characterized histologically as hepatocyte ballooning (*E*). Ultimately, the hepatic inflammatory response drives NASH disease progression as it promotes activation of hepatic stellate cells (HSC) and fibrosis (*F*).

surgery literature. Bariatric surgery leads to restoration of energy balance and improvements in metabolic homeostasis resulting in amelioration of steatosis and marked downstream improvements in, or resolution of, NASH in people. Meta-analysis of multiple bariatric surgery studies demonstrates that this improvement in steatosis observed in 92% of patients is also accompanied by improved steatohepatitis in 81%, lower fibrosis in 66%, and complete resolution in 70% of patients with NASH.[22] Similarly, weight loss in patients with NASH produced by intense nutritional counseling has also been reported to reduce steatosis leading to improvements in NASH resolution.[23] Emerging data also suggest that at least some pharmacological agents that principally target metabolic drivers of steatosis lead to downstream improvements in NASH resolution or fibrosis when administered to patients with NASH with fibrosis, making this an attractive therapeutic angle (vide infra and see Table 1).

Regulators of Hepatic Lipid Metabolism

Over nutrition, accompanied by hyperinsulinemia and hyperglycemia, drives steatosis by promoting de novo lipogenesis (DNL). Lipogenic transcription factors including carbohydrate response element binding protein (ChREBP) and sterol regulatory element binding protein-1c (SREBP-1c) are upregulated in rodent models, leading to increased expression of lipogenic genes and subsequent increased flux through acetyl-CoA carboxylase (ACC) and elevations in hepatic malonyl-CoA.[24] This leads to increased hepatic DNL and suppressed hepatic fatty acid oxidation which together promote steatosis (Figure 1).[24] Hepatic SREBP1c has also been reported to be upregulated in patients with NAFLD[25,26] and elevated rates of hepatic DNL have been found to be a distinctive characteristic of NAFLD.[27] Humans with elevated liver fat showed a >3-fold increase in hepatic DNL relative to subjects with normal liver fat, but differences between the groups were not detected in adipose free fatty acid (FFA) flux or in production of very low-density lipoprotein (VLDL) from FFAs.[27] The observation that fructose consumption, which strongly promotes hepatic DNL,[28] is a risk factor for NAFLD[29,30] may further underscore the importance of DNL in this disease.

Fructose-induced hepatic lipogenesis also likely contributes to the rise in rates of NAFLD. Fructose is a simple sugar found naturally in fruit and is a major component of sucrose (table sugar) and high-fructose corn syrup. Dietary trends in recent decades demonstrate a sharp increase in consumption of fructose and epidemiological studies have shown a strong correlation between consumption of fructose-sweetened beverages and risk for fatty liver.[30–33] Unlike glucose, whose metabolism by the liver is tightly regulated, fructose is rapidly phosphorylated by ketohexokinase to fructose-1-phosphate without feedback inhibition, and is metabolized further in the cell to generate substrates for gluconeogenesis and de novo lipogenesis. Fructose is a more potent inducer of DNL than glucose[34] and chronic fructose consumption induces activation of SREBP1c and ChREBP and downstream lipogenic gene transcription.[31] Thus, strategies aimed at directly inhibiting fructose metabolism (ie, ketohexokinase inhibition[35] or limiting fructose consumption in the diet) represent attractive therapeutic approaches for NAFLD and NASH.

Emerging data also indicate that DNL may be important in regulating some inflammatory pathways in NASH. Berod et al[36] reported that proinflammatory interleukin-17 secreting T cells of the T helper 17 lineage show increased dependency on DNL to produce phospholipids for cellular membranes. In contrast, anti-inflammatory

Table 1. Mechanisms of Action and Associated Pharmacotherapies With Clinical Data in NAFLD/NASH

Mechanism of action	Drug name	Current development status	Key clinical data summary	ClinicalTrials.Gov identifier for key studies
Lipid metabolism pathway modulators				
ACC inhibitor	PF-05175157 (SM)	Ph2—discontinued	Systemically distributed, DNL inhibition, and stimulation of fatty acid oxidation in healthy volunteers. Discontinued due to undisclosed safety concern	NCT01792635
	MK-4074 (SM)	Ph1—discontinued	Liver directed exposure, Hepatic DNL inhibition and steatosis lowering in NAFLD subjects; discontinued due to TG elevations	NCT01431521
	GS-0976 (SM)	Ph2	Liver directed exposure, DNL inhibition, and steatosis lowering in NAFLD patients	NCT02856555
	PF-05221304 (SM)	Ph2	Liver directed exposure, DNL inhibition, no TG increase in healthy subjects at doses which inhibit DNL $\leq$80%	NCT03448172 NCT03248882
FAS inhibitor	TVB-2640 (SM)	Ph2	DNL inhibition in healthy subjects	None listed
DGAT2 inhibitor	IONIS-DGAT2$_{rx}$ (ASO)	Ph2	Ph1 dose-escalation study completed in healthy overweight subjects	NCT03334214
	PF-06427878 (SM)	Ph1 -discontinued	Steatosis lowering in overweight subjects—discontinued due to human PK profile	NCT02855177
	PF-06865571 (SM)	Ph1b/Ph2	None disclosed	NCT03513588 NCT03776175
Nuclear hormone receptor agonists				
PPARγ agonist	Pioglitazone (SM)	Approved T2D, exploratory studies for NASH	Improvements in steatosis, NAS, NASH resolution, and fibrosis	NCT00063622 NCT00994682
PPARα agonist	Fenofibrate (SM)	Approved for dyslipidemia, exploratory studies in NAFLD/NASH	No apparent direct improvements in NASH endpoints or steatosis in small exploratory studies	NCT00262964 NCT02354976
PPARδ agonist	GW501516 (SM)	Ph2—discontinued	Steatosis lowering in subjects with NAFLD	NA
PPARα/δ agonist	Elafibranor (SM)	Ph3	Failed to meet protocol specified primary endpoint in Ph2, modest improvement relative to placebo in post hoc definition of NASH resolution, improvement in cardio metabolic parameters noted	NCT01694849 NCT02704403
PPARα/γ agonist	Saroglitazar (SM)	Approved for dyslipidemia in T2D patients in India, Ph2 for NASH	NAFLD/NASH data pending	NCT03061721

Table 1. Continued

Mechanism of action	Drug name	Current development status	Key clinical data summary	ClinicalTrials.Gov identifier for key studies
FXR	Obeticholic acid (SM)	Conditional approval for PBC, Ph3 for NASH and cirrhosis as a result of NASH	Improvements in all components of NAS, NASH resolution and fibrosis, mild elevations in LDL, reductions in HDL, increased incidence of pruritus	NCT01265498 NCT02548351 NCT03439254
	GS-9674 (SM)	Ph2	Reduction in steatosis and increased incidence of pruritus at 100-mg dose; 30-mg dose (currently being evaluated in ongoing Ph2 monotherapy and combination Ph2 biopsy study) produced very modest relative liver fat reduction (3.7%, placebo adjusted)	NCT02854605 NCT03449446
	Tropifexor (LJN-452) (SM)	Ph2	Interim results of an ongoing Ph2 study were presented noting ALT reductions and modest liver fat content reduction at 60- and 90-μg doses, mild increase in LDL, and decrease in HDL. Increased pruritus incidence relative to placebo noted at 90-μg dose	NCT02855164
	EDP-305 (SM)	Ph2	FGF19 increases and C4 decreases noted in Ph1. Increased incidence in Pruritus and decreased HDL (but no LDL increase) at 20-mg dose relative to placebo	NCT02918929 NCT03421431
	MET409 (SM)	Ph1	Ph1 ongoing	Not posted
THR-β agonist	MGL-3196 (SM)	Ph2	Reduction in liver fat content and ALT noted following 12 and 36 wk of administration. NASH w and $\geq$1-point improvements in fibrosis noted after 36 wk administration.	NCT02912260
	VK2809 (SM)	Ph2	>50% relative reduction (placebo adjusted) in liver fat content noted at 10-mg daily dose. Increases in ALT noted in Ph1 and at early time points in Ph2, though ALT was not different from placebo after 12 wk administration. ALT reduction would have been expected given robust liver fat reductions.	NCT02927184
FGF peptide mimetics				
FGF19	NGM 282 (P)	Ph2	57% and 45% relative reductions (placebo adjusted) in hepatic steatosis noted at 6 and 3-mg doses accompanied by improvements in ALT, AST, and C4. Increased incidence of injection site reactions and GI AEs noted in subjects who received MGM 282 vs placebo. Improvements in all components of NAS as well as fibrosis noted in non–placebo-controlled 12-wk exploratory study.	NCT01943045 NCT02443116
FGF21	BMS-986036 (P)	Ph2	Reductions in liver fat content accompanied by improvements in ALT, AST, Pro-C3, and MRE noted in 16 Ph2 studies. Improvements in cardio metabolic parameters (TG, LDL, HDL) also noted.	NCT02413372

Table 1. Continued

Mechanism of action	Drug name	Current development status	Key clinical data summary	ClinicalTrials.Gov identifier for key studies
β-Klotho/FGFR1c receptor agonist	NGM313 (MK-3655)		Ph1b proof of concept study in normal healthy overweight/obese shows reduction in liver fat content	NCT02708576 NCT03298464
Incretin therapies				
GLP-1 mimetics	Liraglutide (P)	Approved for T2D, exploratory studies for NASH	Improvements in NASH resolution and fibrosis noted in NASH patients administered liraglutide vs placebo for 48 wk.	NCT01237119
	Semaglutide (P)	Approved for T2D, in Ph2 for NASH	Robust improvements in glycemic control and body weight loss noted in both once-weekly and once-daily administered semaglutide. Ph2 biopsy study underway	NCT02970942
DPP4 inhibitor	Sitagliptin (SM)	Approved for T2D, exploratory studies for NASH	Sitagliptin showed no improvements in liver fat content, ALT, AST, and MRE vs placebo in a double-blinded, placebo-controlled study, though some earlier, small, open-label, or retrospective studies showed apparent improvements in liver enzymes and reduction in NAS	NCT01963845
SGLT2 inhibitors				
SGLT1/2 inhibitor	LIK066	Ph2	Ph2 study in obese patients with NASH ongoing	NCT03205150
SGLT2 inhibitor	dapagliflozin	Ph3	Ph3 planned to study efficacy and safety of dapagliflozin in NASH	NCT03723252
Other mechanisms of action				
MPC inhibitor	MSDC-0602K (SM)	Ph2	A press release from Cirius reported that statically significant reductions in ALT and AST were observed in an interim analysis on an ongoing Ph2b trial	NCT02784444
	PXL065 (DRX-065) (SM)	Ph1	Ph1 study in healthy subjects ongoing	Not posted

ACC, acetyl-CoA carboxylase; ALT, alanine aminotransferase; ASO, antisense oligonucleotide; AST, aspartate aminotransferase; DGAT, diacylglycerol O-acyltransferase; DNL, de novo lipogenesis; DPP4, •••; FAS, fatty acid synthase; FGF, fibroblast growth factor; FXR, Farnesoid X receptor; GLP-1, glucagon-like peptide-1; HDL, high-density lipoprotein; MPC, mitochondrial pyruvate carrier; MRE, •••; NAFLD, nonalcoholic fatty liver disease; NAS, Nonalcoholic Fatty Liver Disease Activity Score; NASH, nonalcoholic steatohepatitis; P, peptide or modified peptide; PBC, primary biliary cholangitis; PPAR, peroxisome proliferator-activated receptor; SGLT2, sodium glucose co-transporter 2; SM, small molecule; T2D, type 2 diabetes; TG, triglyceride; THR, thyroid hormone receptor.

regulatory T cells (Treg) utilize exogenous fatty acids. Inhibition of DNL in primary T cells blunts formation of proinflammatory interleukin-17 secreting T cells of the T helper 17 lineage cells and promotes formation of anti-inflammatory Treg. Further, a higher frequency of Th17 cells in liver and an increase in the ratio of Th17 cells to Treg in peripheral blood and in liver marks progression from NAFLD to NASH in humans.[37] Pharmacologic agents that inhibit DNL have potential to reduce steatosis and lipotoxicity. Inhibitors of the lipogenic enzymes ACC, fatty acid synthase (FAS), and diacylglycerol O-acyltransferase (DGAT) are in clinical development for NASH (Table 1).

ACC Inhibitors

ACC catalyzes the first committed step in DNL, the production of malonyl-CoA though the adenosine triphosphate–dependent condensation of acetyl-CoA with carbonate.[38] Malonyl-CoA is also an allosteric inhibitor of carnitine palmitoyltransferase 1, which is limiting for transport of long-chain fatty acyl-CoAs into the mitochondria for beta-oxidation.[38] As a result, ACC acts as a key switch regulating the transition from fatty acid synthesis to fatty acid oxidation. There are 2 closely related isoforms of ACC, ACC1 and ACC2, encoded by separate gene products. In general, ACC2 is the major isoform in oxidative tissues such as muscle and heart, while ACC1 predominates in lipogenic tissues such as liver and adipose; however, ACC2 may be the major ACC isoform in human liver.[39] The first potent and selective small molecule dual ACC1/2 inhibitor, CP-640186, was reported in 2003.[40] Pharmacologic ACC inhibition was shown to inhibit formation of malonyl-CoA, stimulate fatty acid oxidation and block DNL both in vitro and in vivo.[40–45] This compound was the starting point for optimization that ultimately led to the discovery of PF-05175157,[44] a systemically distributed, CNS excluded, dual ACC1/2 inhibitor, which was progressed into clinical development in 2010 (NCT01274663). In Phase 1 studies, PF-05175157 inhibited hepatic DNL and stimulated fatty acid oxidation (as assessed using indirect calorimetry) in human subjects.[44] Clinical development of PF-05175157 was subsequently terminated due to an undisclosed safety finding (NCT01792635). Liver-directed ACC inhibitors presently in development are anticipated to have an improved safety profile relative to the first-generation systemically distributed ACC inhibitor PF-05175157 . In 2017, Kim et al[46] reported that a liver directed ACC inhibitor MK-4074, at doses that fully inhibited hepatic DNL, reduced hepatic steatosis by 44.6% from baseline (placebo adjusted) after 4 weeks of administration. However, MK-4074 unexpectedly increased circulating TG levels 200%.[46] Mechanistic studies in rodents demonstrated that decreases in polyunsaturated fatty acids increased SREBP-1C activation, hepatic GPAT1 expression, and VLDL secretion.[46] Subsequent studies[47] suggest that decreased TG clearance may also contribute to increased circulating TG levels.

A second-generation liver-directed ACC inhibitor, PF-05221304, was also reported to increase serum TG levels in healthy human subjects at doses that inhibited hepatic DNL by $\geq$90%.[48] However, no increases in serum TGs were observed at doses that partially inhibited hepatic DNL by $\leq$80%.[48] These data suggest that at doses that partially inhibit DNL, rather than ablate ACC activity, compensatory increases in circulating TG levels may not be observed. Importantly, 50 to 70% inhibition of DNL is expected to fully normalize the 2- to 3-fold increase in hepatic DNL flux observed in patients with NAFLD.[27] Additional studies are needed to determine if this degree of DNL inhibition can be achieved without circulating TG increases in NASH patients. GS-09674 (formerly NDI-010976) and PF-05221304 are liver directed ACC inhibitors in Phase 2 development for NASH (Table 1). In an open-label non–placebo-controlled study, GS-09674 (20 mg daily) inhibited hepatic DNL by 23% from baseline and reduce steatosis from baseline by 43% after 12 weeks of administration.[49] In a larger randomized, placebo-controlled study, 21% relative liver fat reduction (placebo adjusted, $P = .002$), was observed at the 20-mg daily dose after 12 weeks of administration.[50]

FAS Inhibitors

FAS, a multidomain enzyme complex, catalyzes the production of palmitate using malonyl-CoA, acetyl-CoA, and nicotinamide adenine dinucleotide phosphate as an electron donor. FAS inhibitors have been of interest for oncology as many tumor types depend on increased DNL flux for membrane production and to maintain redox balance by regenerating NADP+.[51,52] Early generation FAS inhibitors suffered from 1 or more liabilities including poor potency, off target activity, or suboptimal physiochemical or pharmacokinetic properties.[52,53] While early FAS inhibitors functioned as substrate competitors, more recently inhibitors targeting co-factor binding sites have been identified. TVB-2640 is the first FAS inhibitor to enter clinical development. TVB-2640 inhibited DNL in human subjects with metabolic syndrome.[54] TVB-2640 is in Phase 1 development for NASH (Table 1) and Phase 2 development for oncology.

DGAT1 and DGAT2 Inhibitors

DGATs catalyze the esterification of a fatty acid with diacylglycerol to form TG.[55] DGAT activity contributes to intestinal fat absorption, control of circulating lipid concentrations, flux of lipoproteins between adipose and liver, and muscle energy metabolism.[56] In mammals, 2 DGAT enzymes have been characterized; DGAT1 is highly expressed in intestine and plays a central role in fat absorption[57] while DGAT2 is highly expressed in liver and adipose tissue.[58] Within hepatocytes, DGAT1 primarily esterifies exogenous fatty acids while DGAT2 primarily incorporates DNL-derived fatty acids into TG.[59] Pharmacologic inhibitors of DGAT1 and DGAT2 have been evaluated in nonclinical models and progressed into clinical development. Small molecule DGAT1 inhibitors of multiple structural classes block postprandial TG excursion following lipid challenge in nonclinical models.[60] Pronounced and dose-limiting GI side effects were observed with DGAT1 inhibitors in human subjects leading to compound discontinuation.[60–63] In contrast, DGAT2 is not expressed at high levels in the intestine[58] and does not play a major role

in intestinal lipid absorption.[64] Anti-sense oligonucleotide (ASO) knock down and pharmacological inhibition of DGAT2 suppresses TG production, but also induces adaptive downregulation of SREBP1, leading to decreased lipogenic gene expression and induction of oxidative pathways.[65,66] Thus, DGAT2 inhibition exerts both direct inhibition of hepatic TG synthesis and adaptive suppression of DNL and stimulation of hepatic fatty acid oxidation. The net result of these changes is to reduce hepatic diacylglycerol and TG which, in turn, lowers hepatocyte lipid and VLDL-TG secretion in rodents.[65,66] Similarly, oral administration of a small molecule DGAT2 inhibitor PF-06427878 for 2 weeks reduced steatosis, as assessed using magnetic resonance imaging proton density fat fraction (PDFF), by ~40% from baseline (placebo adjusted) in human NAFLD subjects.[67] This was accompanied by improvements in liver enzymes.[67] Yamaguchi et al[68] reported, however, that DGAT2 ASO administration to db/db mice fed a methionine- and choline-deficient diet reduced steatosis but increased fibrosis relative to mice treated with saline. However, in more recent studies, small molecule DGAT2 inhibitors improved fibrosis in the STAM mouse model,[69,70] the choline deficient high fat fed rat model[71] and the LDLr−/− Leiden NASH rat model.[72] Additional experiments are needed to determine if these differences are attributed to the use of ASO vs small molecule inhibitors, the specific ASO used by Yamaguchi et al,[68] direct effects on DGAT2 inhibition or differences in animal models. Currently, a DGAT2 ASO (IONIS-DGAT2$_{Rx}$) is reported to be in Phase 2 (NCT03334214) and the small molecule DGAT2 inhibitor PF-06865571 is in Phase 2 (NCT03776175) development for NASH as monotherapy and in combination with PF-05221304 (Table 1).

Nuclear Hormone Receptors

Nuclear hormone receptors act as ligand-inducible transcription factors to exert pleiotropic effects on metabolism[73,74] and thus may exert pleiotropic effects. Nuclear hormone receptor modulators are of interest for NASH. Specifically, peroxisome proliferator-activated receptor (PPAR) modulators, Farnesoid X receptor (FXR) agonists, and thyroid hormone receptor β (THR-β) agonists have been evaluated in NAFLD or NASH trials or are presently in development for NASH (Table 1). Two investigational agents claimed to exert the NASH benefits of pioglitazone independent of activity on PPARγ, are also in clinical development for NASH (Table 1).

Peroxisome Proliferator-Activated Receptors

PPARs are members of the nuclear hormone receptor subfamily that act as ligand-activated transcription factors to regulate metabolic and energy homeostasis. There are 3 PPAR subtypes with distinct tissue distribution and physiological function: PPARα, PPARγ, and PPARβ/δ. PPARα has relatively high expression in the liver and activation reduces hepatic triglyceride levels. Activation of PPARγ improves insulin sensitivity and enhances glucose metabolism, while activation of PPARβ/δ promotes fatty acid metabolism and suppresses macrophage-mediated inflammation.[75] Pioglitazone (Table 1) is a thiazolidinedione (TZD) insulin sensitizing PPARγ agonist approved for glycemic control in T2D patients. Multiple studies have evaluated the effects of pioglitazone on NASH biopsy endpoints in patients with T2D and, in some cases, those without.[76] In the Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis (PIVENS) trial, pioglitazone (30 mg daily) produced reductions in steatosis ($P < .001$), lobular inflammation ($P = .004$), but not fibrosis score ($P = .12$) relative to placebo in patients with NASH without T2D.[77] However, the primary outcome, which assessed a standardized composite score for steatosis, lobular inflammation, hepatocyte ballooning, and fibrosis was not met ($P = .04$ vs target value of $P < .025$).[77] Cusi et al[78] evaluated administration of pioglitazone (45 mg daily) or placebo along with a hypocaloric diet for 18 months in NASH patients with prediabetes or T2D. 58% of subjects whom received pioglitazone, vs 17% for placebo, achieved the primary endpoint goal (2-point reduction in NAFLD Activity Score [NAS]), and 51%, vs 19% for placebo, demonstrated NASH resolution. Pioglitazone administration was associated with an improvement in fibrosis score. Additionally, a meta-analysis of randomized clinical trials in which patients were treated with pioglitazone or rosiglitazone concluded that TZD administration was associated with improved advanced fibrosis (odds ratio [OR], 3.15; 95% confidence interval [CI], 1.25–7.93; $P = .01$; $I^2 = 0\%$), improved fibrosis of any stage (OR, 1.66; 95% CI, 1.12–2.47; $P = .01$; $I^2 = 0\%$), and NASH resolution (OR, 3.22; 95% CI, 2.17–4.79; $P < .001$; $I^2 = 0\%$).[76] The observation that TZDs, and notably pioglitazone, led to improvements in multiple dimensions of NASH supports that hypothesis that agents that address the metabolic underpinnings of NASH can lead to downstream improvements in NASH resolution and fibrosis.

While pioglitazone is traditionally thought to act as an agonist of PPARγ, others have hypothesized that the benefits of pioglitazone, but not the side effects of the drug, can be separated from PPAR activity.[79,80] These investigators postulated that TZDs attenuate mitochondrial pyruvate carrier activity and that new selective mitochondrial pyruvate carrier modulators will share the therapeutic benefits of TZDs but not the side effects that limit TZD use.[79,80] Two such agents, MSDC-0602K (Cirius Therapeutics, San Diego, CA), and PXL065 (formerly DRX-065 from DeuteRx) (Poxel, Lyon, France) are presently in development for NASH (Table 1). The data generated in these trials will be helpful in testing this hypothesis.

Elafibranor is a dual PPARβ/δ agonist in Phase 3 development for NASH (Table 1). PPARα regulates lipid flux into the liver, regulating hepatic fatty acid uptake and oxidation, resulting in circulating TG reductions and high-density lipoprotein (HDL) increases. Fibrates are PPARα agonists indicted as adjunctive therapy to diet to reduce elevated LDL, total cholesterol, TG and apolipoprotein B, and to increase HDL in adult patients with primary hypercholesterolemia. A small pilot study in patients with biopsy confirmed NASH did not demonstrate significant improvements in NAFLD activity score, steatosis, or fibrosis from baseline measures following 48 weeks administration of

fenofibrate (200 mg daily).[81] Additionally, fenofibrate (200 mg daily) administration did not reduce steatosis in overweight or obese human volunteers with NAFLD, though serum TG reductions were observed.[82,83] These observations suggest that selective PPARα agonists may not contribute directly to NASH efficacy. PPARδ regulates fatty acid uptake and β-oxidation as well as insulin sensitivity.[84] Administration of the PPARδ agonist GW501516 (Table 1) (10 mg daily) to healthy moderately overweight subjects lowered liver fat content by 20% relative to placebo (baseline adjusted) ($P < .05$).[85] GW501516 administration also produced significant reductions in fasting TG (−30%), LDL (−23%), and insulin (−11%).[85]

In the Phase IIb Study to Evaluate the Efficacy and Safety of GFT505 Versus Placebo in Patients With Non-Alcoholic Steatohepatitis (NASH) (GOLDEN-505) Phase 2 trial, elafibranor (Table 1) (80 mg and 120 mg daily) or placebo was administered daily to patients with NASH without cirrhosis for 52 weeks. Elafibranor failed to meet the intent to treat protocol-defined primary outcome, resolution of NASH without fibrosis worsening.[86] However, a modestly higher proportion of patients in the 120 mg elafibranor group (19%) vs the placebo group (12%) achieved a post hoc modified definition of NASH resolution without fibrosis.[86] Elafibranor administration was also associated with improvement in cardiometabolic risk factors including a 0.46% (placebo adjusted) reduction in hemoglobin A1C, and reductions in fasting plasma glucose, homeostatic model assessment of insulin resistance (HOMA-IR), TG, LDL, FFA, fructosamine, and C-peptide.[86] The Phase 3 Study to Evaluate the Efficacy and Safety of Elafibranor Versus Placebo in Patients With Nonalcoholic Steatohepatitis (NASH) (RESOLVE-IT) Phase 3 study [Phase 3 Study to Evaluate the Efficacy and Safety of Elafibranor Versus Placebo in Patients With Nonalcoholic Steatohepatitis (NASH)] is presently underway to evaluate the efficacy and safety of elafibranor versus placebo in patients with NASH (NCT02704403). Saroglitazar (Table 1) is a dual PPARα/γ agonist that is approved in India for treatment of diabetic dyslipidemia and hypertriglyceridemia not controlled by statin therapy.[87] Saroglitazar is also presently in Phase 2 development for NASH (NCT03061721).

FXR Agonists

FXR is a nuclear hormone receptor activated by bile acids.[88] FXR forms a heterodimer with the retinoid X receptor to regulate expression of target genes, generally through binding to inverted repeat elements separated by a single nucleotide.[89] FXR is expressed in the ileum and in hepatic parenchymal and nonparenchymal cells such as Kupffer and stellate cells. FXR has multiple functions including feedback regulation of bile acid synthesis, regulation of glucose and lipid metabolism and regulation of hepatic inflammation and fibrosis.[88] Multiple FXR agonists are in clinical development for NASH (Table 1). These agents are thought to drive efficacy through a combination of intestinal action leading to increased secretion of fibroblast growth factor 19 (FGF19) (or FGF15 in rodents) as well as direct action on the liver.

The most advanced FXR agonist is obeticholic acid, INT-747 (Table 1), a bile acid derivative presently approved for the treatment of primary biliary cholangitis and recently completed a Phase 3 trial (Randomized Global Phase 3 Study to Evaluate the Impact on NASH With Fibrosis of Obeticholic Acid Treatment [REGENERATE]) for the treatment of NASH with fibrosis. In the Phase 2 Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment (FLINT trial), 45% of subjects administered obeticholic acid (25 mg daily) for 72 weeks versus 21% of subjects administered placebo ($P = .0002$) had improved liver histology.[90] Multiple dimensions of NASH pathogenesis were improved with 22% vs 13% ($P = .08$) showing NASH resolution, 35% vs 19% ($P = .004$) showing improvements in fibrosis, 45% vs 31% ($P = .03$) showing improvements in hepatocyte ballooning, 53% vs 35% ($P = .006$) showing improvements in lobular inflammation, and 61% vs 38% ($P = .001$) showing improvements in steatosis, in subjects administered obeticholic acid or placebo, respectively.[90] Administration of obeticholic acid vs placebo was also associated with worsening in multiple cardiometabolic risk factors including significant increases in total cholesterol ($P = .009$), LDL cholesterol ($P < .0001$), alkaline phosphatase ($P < .0001$), HOMA-IR ($P < .01$), and decreased HDL ($P = .01$).[90] In addition, administration of obeticholic acid was associated with increased incidence of pruritus, occurring in 23% of subjects who received obeticholic acid vs 6% of subjects who received placebo.[90] In a subsequent study in Japanese subjects with NASH, significant improvements in the NAS were not observed in subjects administered obeticholic acid for 72 weeks at 10 mg ($P = .807$) or 20 mg ($P = .3378$).[91] The 40-mg dose group achieved statistical significance ($P = .0496$) vs subjects who received placebo with 38% vs 20% of subjects, respectively, showing a 2-point improvement in NASH resolution.[91] Significant differences were not observed in other histology endpoints including the proportion of patients with steatosis and inflammation improvement, ballooning resolution, and NASH resolution. No differences in improvements in fibrosis were observed between subjects who received obeticholic acid or placebo though dose-responsive increases in pruritus and changes in LDL, HDL, and cholesterol were consistent with data reported in the FLINT trial.[91] Reasons for the apparent efficacy differences between efficacy responses in the FLINT trial conducted in the United States and the Japanese Phase 2 study are not clear. In the Phase 3 REGENERATE trial (NCT02548351), 23.1% of patients with NASH and F2 or F3 fibrosis who received 25-mg obeticholic acid daily vs 11.9% of subjects who received placebo ($P = .0002$) demonstrated a $\geq$1 stage improvement in fibrosis without worsening of NASH.[92] There was no significant difference in the proportion of subjects who achieved $\geq$1 stage improvement in fibrosis without worsening of NASH who received 10-mg obeticholic acid daily vs placebo. Additionally, neither the 25-mg dose nor the 10-mg dose achieved significance vs placebo in the proportion of subjects with NASH and F2 or F3 fibrosis that achieved resolution of NASH without worsening of fibrosis.[92] Pruritus was observed in 51% of subjects who received 25-mg obeticholic acid vs 18% of subjects who received placebo and pruritus associated treatment

discontinuation was higher in the 25-mg obeticholic acid treatment group (9%) than placebo (<1%). More patients (3%) in the 25-mg obeticholic acid treatment group experienced gallstones or cholecystitis compared with the placebo group (<1%), and consistent with the results of the Phase 2 FLINT study, obeticholic acid treatment was associated with an increase in LDL cholesterol.[92]

The Food and Drug Administration identified 19 cases of death and 11 cases of serious liver injury in patients being treated obeticholic acid (Ocaliva) for primary biliary cholangitis, resulting in issuance of a Food and Drug Administration warning on September 21, 2017, and an update to this warning on February 1, 2018.[93] Most of these cases were in subjects with moderate-to-severe decreases in liver function who received excessive dosing relative to the doses recommended for patients with impaired liver function. The warning noted that Ocaliva may also be associated with liver injury in some patients with mild disease who are receiving the correct dose, as 5 cases of serious liver injury were reported in patients with no or mild decreases in liver function before initiating Ocaliva.[93] The impact of this warning for the NASH indication is unclear. Phase 3 trials evaluating the efficacy of obeticholic acid (10 and 25 mg daily) in patients with compensated cirrhosis as a consequence of NASH (REVERSE trial; NCT03439254) is ongoing.

While obeticholic acid is a steroidal bile acid analog, other FXR agonists in development are steroidal or nonsteroidal non–bile acid derivatives. These second generation FXR agonists were initially hypothesized to have an improved safety profile relative to obeticholic acid, though emerging clinical data suggest that the pruritus and worsened cardiometabolic risk factor response associated with obeticholic acid administration may be a consequence of FXR agonism rather than off-target effects resulting from the bile acid or steroidal structure of the compound. Administration of the non–bile acid, nonsteroidal FXR agonist GS-9674 (Table 1) produced a 24.6% relative median reduction in liver fat (placebo adjusted) at the 100-mg dose ($P < .001$), but only a 3.7% relative median reduction in liver fat (placebo adjusted) at the 30-mg dose ($P = .029$) in a Phase 2 study in NASH patients.[94] These reductions in steatosis were associated with trends toward reductions in alanine aminotransferase (ALT), though these did not reach statistical significance ($P = .11$ and $P = .41$ for 100 mg and 30 mg, respectively).[94] Trends toward increased total cholesterol (5% [$P = .14$] and 4.4% [$P = .48$] for 100 mg and 30 mg, respectively, placebo adjusted) and LDL cholesterol (10.2% [$P = .13$] and 9.8% [$P = .28$] for 100 mg and 30 mg, respectively) were also observed. Moderate to severe pruritus was observed in 14.3% of subjects who received 100 mg GS-9674, compared with 3.6% of subjects who received 30 mg GS-9674 or placebo.[94] At the time of writing, only the 30-mg dose of GS-9674, which produced only a modest 3.7% placebo-adjusted reduction in steatosis, is being evaluated in the ongoing Phase 2 Safety and Efficacy of Selonsertib, Firsocostat, Cilofexor, and Combinations in Participants With Bridging Fibrosis or Compensated Cirrhosis Due to Nonalcoholic Steatohepatitis (NASH) (ATLAS) trial investigating the safety, tolerability, and efficacy of selonsertib, GS-0976, and GS-9674 as monotherapy or in combination (co-administration) in NASH patients (NCT02781584).

Interim results were presented from the Study of Safety and Efficacy of Tropifexor (LJN452) in Patients With Nonalcoholic Steatohepatitis (NASH) FLIGHT-FXR Phase 2 study (NCT02855164) evaluating safety and efficacy of tropifexor (LJN-452) in NASH patients.[95] Liver fat content was reduced by 5.4% and 10.7% (relative reduction from baseline, placebo adjusted) at the 60-μg and 90-μg doses, respectively. Similarly, ALT was reduced from baseline by 8.2% and 11.4% (placebo adjusted) at the 60-μg and 90-μg doses, respectively. Tropifexor administration was associated with a mild increase in LDL and decrease in HDL and pruritus was reported in 14% and 8% of subjects at the 60-μg and 90-μg doses, respectively, relative to 7% of subjects that received placebo.

EDP-305 (Table 1) a steroidal, non–bile acid, FXR agonist is being evaluated in a Phase 2 study to assess the safety, tolerability, pharmacokinetics and efficacy in NASH patients (NCT03421431). In a Phase 1 multiple ascending dose study, robust FXR target engagement was demonstrated for EDP-305 with increases in FGF19 and decreases in C4.[96] Increased incidence of pruritus was observed in subjects who received the 20-mg dose of EDP-305 relative to placebo.[96] This dose was also associated with reductions in HDL and total cholesterol, but not increases in LDL, in subjects with presumptive NASH.[96]

THR-β Agonists

The thyroid hormone (TH) triiodothyronine and its prohormone thyroxine exert beneficial metabolic effects on TG and cholesterol through action on the THR-β in the liver. However, TH also exerts adverse cardiac and bone effects primarily through action on THR-α. First-generation THR-β–selective agonists sobetirome (GC-1) and eprotirome (KB2115) demonstrated marked reductions in circulating lipids, but were terminated for poor toleration (sobetirome) or significant elevation in transaminases in humans and deleterious cartilage findings in nonclinical toxicology studies (eprotirome).[97]

MGL-3196 is liver directed THR-β selective agonist designed to minimize action outside the liver by improved selectivity vs THR-α and selective uptake into the liver.[98] MGL-3196 administration to healthy volunteers with mildly elevated cholesterol was well tolerated with reductions in total cholesterol, LDL and TG levels observed at doses >50 mg.[99] A phase 2 study was conducted evaluating MGL-3196 in NASH patients utilizing an atypical dosing regimen where MGL-3196 was administered starting at 80 mg with a subject by subject ±20-mg dose adjustment possible at week 4 based on individual subject exposure.[100] This atypical dosing regime was likely utilized because MGL-3196 demonstrated variability in exposure at higher doses and a ~3-fold increase in exposure in healthy subjects at 100 mg relative to exposures observed at 80 mg after 14 days of dosing.[99] After 12 weeks of administration, MGL-3196 produced a 26.7% relative reduction in liver fat

from baseline (placebo adjusted) and a 5.8% relative reduction in ALT from baseline (placebo adjusted).[100] After 36 weeks of administration, the reduction in liver fat was sustained (29% relative reduction in liver fat from baseline, placebo adjusted).[101] In addition, NASH resolution was observed in 27% of subjects administered MGL-3196 vs 6% of subjects who received placebo ($P = .02$).[101] Resolution in NASH was principally observed in those MGL-3196 treated subjects who achieved ≥30% relative reduction from baseline of liver fat content. 37% of the patients who received MGL-3196 and showed ≥30% relative reduction from baseline in liver fat content achieved NASH resolution, while only 4% of patients who received MGL-3196 and did not achieve this level of liver fat reduction demonstrated NASH resolution. Further, ≥1-point reduction in fibrosis was observed in 32% of subjects who received MGL-3196 vs 12% of subjects who received placebo ($P = .03$).[101] A second THR-β selective agonist VK2809 (formerly MB 07811) is also in Phase 2 development for NASH. In a 12-week Phase 2 study in patients with NAFLD, subjects who received 10-mg daily VK2809 administration showed a 50.8% ($P < .01$) reduction from baseline in liver fat content (placebo adjusted).[102] ALT levels increased, mostly during early treatment, but after 12 weeks of administration, ALT levels were not different in subjects that received VK2809 or placebo. ALT increases were also observed with VK2809 in some subjects in Phase 1.[103]

Adipose Tissue Metabolism in Nafld

Obesity and systemic insulin resistance are major comorbidities associated with NAFLD[104,105] and human studies demonstrate that circulating FFAs derived from adipose tissue lipolysis are the principal supplier of fat to the liver and the single biggest contributor to fasting VLDL-TG in human NAFLD.[27,106,107] An important study in humans by Lambert et al[27] used stable isotopes to compare fatty acid flux in subjects with NAFLD compared with controls matched for other metabolic parameters, and demonstrated that NAFLD subjects not only have elevated hepatic de novo lipogenesis, but also have significantly elevated nocturnal plasma FFA levels. Given that most of the fat in the liver is derived from adipose tissue lipolysis, this could be an important target for NAFLD pharmacotherapy. In healthy lean people, insulin normally inhibits the breakdown and release of fatty acids from adipocytes. As insulin resistance develops in the context of metabolic disease, however, there is a failure of insulin to suppress hormone sensitive lipase, leading to a largely unrestrained release of FFAs from adipose tissue into the blood. The majority of these circulating FFAs are subsequently taken up by the liver resulting in hepatic steatosis. In addition, hypertrophy of expanding adipocytes leads to recruitment of adipose tissue macrophages and release of inflammatory cytokines including tumor necrosis factor alpha. Tumor necrosis factor alpha further exacerbates adipose insulin resistance via direct effects on the insulin receptor signaling cascade, leading to additional effects on lipolysis.[108] The relative contribution of distinct adipose depots to NAFLD pathogenesis in humans is largely unknown. Visceral adipose tissue lies anatomically adjacent to the portal vein, is a major source of inflammatory cytokines and is closely linked to systemic insulin resistance yet contributes a relatively small percentage to the overall nonesterified fatty acid pool. Recent studies have implicated abdominal subcutaneous adipose tissue dysfunction in NASH lipotoxicity, pointing to likely contributions from multiple adipose depots in linking systemic insulin resistance to NAFLD.[109] Overall there is a compelling body of evidence linking dysfunctional adipose tissue metabolism to NASH subjects,[110] suggesting a path forward for NASH treatments that improve adipose tissue health, restore adipose insulin sensitivity and inhibit adipose tissue lipolysis.

Glycemic Modulators

Incretin Therapies

Glucagon-like peptide-1 (GLP-1) is a gut-derived incretin hormone with pleiotropic beneficial metabolic effects, including stimulation of insulin secretion, inhibition of glucagon secretion, and weight loss.[111] Incretin-based therapies are increasingly being used to treat T2D and obesity. Given the efficacy of GLP-1R agonists and DPP-4 inhibitors in treating insulin-resistant metabolic dysfunction, it is no surprise that these mechanisms are also being investigated as potential NASH therapies. Numerous GLP-1 analogs, GLP-1R agonists, and DPP-4 inhibitors have shown promising efficacy in many preclinical models of NAFLD and NASH (reviewed in Bifari et al[112]). Human subjects with insulin resistance and NAFLD have been shown to have increased hepatic messenger RNA expression of DPP-4[113] and slight elevations in plasma DPP-4 activity compared with healthy control subjects.[114] However, the DPP-4 inhibitor sitagliptin showed no beneficial effect on insulin resistance or liver steatosis/function in small clinical trials.[115,116] The most compelling evidence to date for the potential of the GLP-1 mechanism in NASH arises from the LEAN (Liraglutide Efficacy and Action in NASH) trial. The GLP-1R agonist liraglutide (dose = 1.8 mg given once daily for 48 weeks to overweight and obese subjects with a diagnosis of NASH) resulted in increased resolution of NASH and importantly, reduced progression of fibrosis, compared with the placebo group.[117] The longer acting GLP-1 agonist semaglutide is currently being evaluated in a Phase 2 trial (NCT02970942) for subjects with biopsy-confirmed NASH.

Agonism of the glucagon receptor is also being evaluated for efficacy in NAFLD/NASH, primarily in combination with GLP-1 agonists, due to expected effects on reducing food intake and increasing energy expenditure. Additionally, dual glucose-dependent insulinotropic polypeptide/GLP-1 receptor agonists are also under development for treating metabolic dysfunction; this dual incretin approach appears to be more efficacious on metabolic endpoints preclinically when compared with single agonists (reviewed in Seghieri et al[118]). In support of this concept, recent results published from a randomized, placebo-controlled Phase 2 trial of the dual glucose-dependent insulinotropic polypeptide/GLP-1R

agonist LY3298176 in patients with T2D demonstrated statistically significant HbA1c lowering with greater weight loss than the GLP-1R agonist dulaglutide alone.[119] Furthermore, the polypeptide SAR425899, which acts as an agonist at both the GLP-1R and the glucagon receptor, reduced body weight and lowered glycemia in type 2 diabetes mellitus (T2DM) subjects,[120] and is currently being investigated for efficacy in T2DM patients with NASH (NCT03437720). Numerous agents are currently under development in this area for their promise to reduce body weight and subsequently impact insulin resistance and liver health (summarized in Brandt et al[121]).

The mechanism(s) by which GLP1 improves hepatic steatosis, liver inflammation, and injury remain unclear. Although somewhat controversial, the liver is not likely a direct target of GLP1's actions because most studies have not been able to detect expression of the canonical GLP1 receptors in hepatocytes.[122,123] It is possible that improved steatosis seen with GLP1-based therapies is secondary to weight loss, improved glycemic control and effects on inflammation or the gut microbiota. Regardless, a treatment that targets multiple components of metabolic derangement present in NASH, including body weight, glycemia, hepatic steatosis and inflammation, is highly desirable. Furthermore, the reduced cardiovascular risk shown in the LEADER (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results) trial provides additional support for this mechanism in treating NASH patients who are at higher risk of cardiovascular disease.[124] Although incretin-based therapies for NASH appear promising and will continue to be vetted in the clinic, more studies are needed to gain an understanding of the relevant site(s) of action and a clear mechanistic link to disease resolution.

Sodium Glucose Co-transporter 2 Inhibitors

Insulin resistance and NAFLD are tightly linked as evidenced by the significantly increased risk of NAFLD in patients with T2D.[125] Inhibitors of the renal sodium glucose co-transporter 2 (SGLT2) are a growing class of T2DM agents which improve blood glucose levels by increasing urinary output of excess glucose (reviewed in Vivian[126]). Treatment with SGLT2 inhibitors leads to multiple beneficial effects, including improved glycemia without risk of hypoglycemia, improved cardiovascular risk factors, and slight reductions in body weight. The class in general is well-tolerated, with urinary tract and genital infections being the most common side effects, although more serious adverse events including euglycemic diabetic ketoacidosis[127] and increased risk of lower limb amputation have been reported for at least 1 member of the SGLT2 inhibitor class.[128] Notably, results of the EMPA-REG clinical trial (NCT01131676) demonstrated that administration of once-daily empagliflozin in patients with T2D at high cardiovascular risk resulted in a reduction in cardiovascular mortality compared with placebo.[129] Given that NASH patients are at an increased risk of cardiovascular mortality,[125] this is an attractive feature for potential NASH pharmacotherapy.

Numerous preclinical studies have demonstrated benefit of SGLT2 inhibition in NASH models.[11,130–135] To date, however, only a few relatively small clinical trials have been conducted with SGLT2 inhibitors in NAFLD or NASH patients (summarized in Khan et al[125]). It is difficult to draw robust conclusions from these studies due to the small sample size and mild effects on most endpoints examined. In a small study on the effects of canagliflozin on hepatic function in T2DM patients with biopsy-confirmed NASH (n = 10), subjects received 100-mg canagliflozin once a day for 12 weeks. A mild degree of ALT lowering was reported: the change in ALT from baseline to week 12 was −23.9 U/L (95% CI, −48.1 to 0.3; $P = .0526$).[136] Empagliflozin was found to lower ALT/aspartate aminotransferase (AST) levels in individuals with T2D assessed by analysis of several placebo-controlled randomized trials including the EMPA-REG OUTCOME trial.[137] In the E-LIFT trial, 50 patients with T2DM and NAFLD were randomly assigned to the control group or to receive 10-mg daily empagliflozin for 20 weeks with a change in liver fat measured by magnetic resonance imaging PDFF. When compared with baseline measurements, the empagliflozin group showed a statistically significant reduction in magnetic resonance imaging PDFF (16.2% to 11.3%; $P < .0001$) while the control group did not show a statistically significant reduction (16.4% to 15.5%; $P < .057$).[138] Several open-label pilot studies are listed in ClinicalTrials.gov investigating SGLT2 inhibitors in NAFLD patients (NCT02696941; NCT02875821; NCT02964715; NCT02649465; NCT03646292), and a prospective study examining the effects on empagliflozin on liver fat and energy metabolism in patients with newly diagnosed T2DM recently completed in August 2018 (NCT02637973). Currently, a combined SGLT1/2 inhibitor (LIK066) is being investigated in a 12-week randomized, placebo-controlled study in obese subjects with NASH (NCT03205150). Finally, the SGLT2 inhibitor dapagliflozin will be evaluated in a randomized, placebo-controlled phase 3 interventional trial (NCT03723252) and is expected to be completed in 2021.

The precise mechanism for improvements in NAFLD or NASH pathogenesis with the SGLT2 inhibitor class is likely multifactorial. SGLT2 inhibition leads to a small amount of weight loss, improvements in glycemic control, reduced insulin levels and a reduction in inflammation. Lower circulating glucose and insulin levels result in decreased activation of ChREBP and SREBP1c in the liver and subsequently reduced fatty acid synthesis. Regardless of the mechanism, initial small trials hint at possible efficacy for this class of drugs in improving NAFLD, an established comorbidity in diabetic patients. Larger clinical trials with hepatic histopathological endpoints are needed to establish efficacy in treating NASH.

Fibroblast Growth Factors

FGFs comprise a large, pleiotropic family of growth factors implicated in a broad range of physiological processes including cell growth and differentiation and metabolic control. FGFs can act in an autocrine, paracrine, or endocrine manner, and signaling is initiated by binding to an FGF receptor and co-receptor (ie, β-klotho).[139] FGF21 is produced in the liver and other metabolically active tissues, and

in rodents is nutritionally regulated as a target of PPARα.[140–142] Depending on the species, pharmacological administration of FGF21 has been shown to improve dyslipidemia, reduce body weight and improve insulin sensitivity, making this a potentially attractive therapy for treatment of metabolic disorders (reviewed in Gimeno and Moller[143], Reitman,[144] and Zhang and Li[145]). The precise mechanism(s) by which FGF21 may improve metabolic dysfunction are largely unknown and are likely pleiotropic, involving multiple cell types or sites of action and downstream metabolic pathways including stimulation of hepatic fatty acid oxidation, glucose uptake into adipose, autonomic regulation in the brain and anti-inflammatory effects.[146–148]

FGF21 has received significant attention as a possible therapeutic modality for NASH due to its demonstrated beneficial effects on liver metabolism in rodents, primates and humans. Increased fructose consumption is associated with fatty liver disease and leads to an elevation of circulating FGF21 levels[149] via a ChREBP-mediated mechanism.[150] In this context, FGF21 is an important component of the protective, homeostatic response to fructose consumption and the absence of FGF21 in mice on a high-fructose diet exacerbates liver disease.[150] Consistent with this notion, a recent study demonstrated that FGF21 is required to blunt progression from fatty liver to hepatocellular carcinoma upon prolonged consumption of a high-fat, high-sucrose diet.[151] Mice deficient in FGF21 develop significant hepatic steatosis, worsening of fibrosis and inflammation and apoptosis on a methionine- and choline- deficient diet which could be improved via FGF21 infusion.[152] Treatment of obese mice with FGF21 leads to weight loss, reduction of hepatic steatosis and improved liver metabolic function[153–159]; importantly, FGF21 administration in NASH rodent models also has body weight-independent anti-inflammatory and antifibrotic effects in the liver.[152] In Zucker rats, FGF21 improved glucose tolerance and liver insulin sensitivity without significant effects on body weight.[160] Consistently, in diabetic rhesus monkeys FGF21 administration led to improvements in fasting plasma glucose and insulin levels, reduced triglycerides with only a minor reduction in body weight,[161] and injection of a FGF21 mimetic antibody activating the β-klotho/FGFR1c receptor in obese cynomolgus monkeys induced reductions in plasma insulin, triglycerides, glucose, and body weight.[162]

Numerous FGF21 analogs and mimetics have been developed and are in various stages of clinical development (Table 1 and summarized in Gimeno and Moller[143]). Phase 1 clinical trials show improvements in dyslipidemia, insulin sensitivity, and body weight in obese/T2D subjects.[163–165] Numerous Phase 2 clinical trials are being conducted with a pegylated form of FGF21 for the treatment of NASH (BMS-986036; A Study of Experimental Medication BMS-986036 in Adults With Nonalcoholic Steatohepatitis (NASH) and Stage 3 Liver Fibrosis (FALCON 1) trials; NCT02413372),[166] and an integrated safety analysis presented at the International Liver Congress (European Association for the Study of the Liver) meeting in April 2018 concluded that daily or weekly subcutaneous dosing of BMS-986036 for up to 16 weeks was generally well tolerated in obese or T2D subjects.[167] Potential safety signals have been reported in some clinical trials using a long-acting FGF21 molecule, PF-05231023, including modulation of markers of bone formation/turnover and increases in blood pressure and pulse rate not predicted in nonhuman primates.[164,165] It is unclear whether these are mechanistic effects of FGF21 agonism; thus, it will be important to do a comprehensive safety assessment of current and future clinical stage FGF21 assets. The interest in this mechanism remains robust as evidenced by recent optioning of the β-klotho/FGFR1c receptor agonist NGM313 (renamed MK-3655[168]), which showed reductions in steatosis in a Phase 1 open label single dose study without a placebo control (NCT03298464),[169] and interest in another FGF21 analog (AKR-001).

Rodent FGF15 and the human analog FGF19 are expressed in enterocytes of the small intestine and secreted into portal circulation. FGF15/FGF19 transcription is regulated by FXR binding to a response element on the *FGF15/19* gene and circulating FGF15/19 levels are increased as a consequence of intestinal FXR agonist activity. FGF15/19 functions to regulate bile acid synthesis as well as carbohydrate metabolism and energy expenditure.[170] FGF15/19 inhibits bile acid synthesis in the liver by repressing transcription of cholesterol 7α-hydroxylase, the rate-limiting enzyme in the bile acid synthetic pathway, and stimulates the gallbladder to fill with bile. FGF15/19 promotes hepatic glycogen and protein synthesis through action on the FGFR4/β-klotho receptor complex in the liver and suppresses hepatic gluconeogenesis. Mice expressing FGF19 have higher metabolic rate, improved glycemic control, and reduced body weight and liver fat content despite increased food consumption. Additionally, however, FGF15/19 also promotes liver growth and has also been implicated in liver tumorigenesis.[170] NGM282 is a modified analog of FGF19 designed to be a nontumorigenic variant that maintains the positive metabolic functions of native FGF19.[171] Once-daily subcutaneous administration of NGM282 for 12 weeks to patients with NASH produced marked reductions in steatosis with a 57% and 45% ($P < .0001$) relative reduction from baseline (placebo adjusted) observed at the 6- and 3-mg doses, respectively.[172] Improvements in ALT ($P < .0001$), AST ($P < .0001$), and C4 ($P < .0001$) were also noted, though improvements in glycemic control were not observed. Injection site reactions were observed in 54% and 41% of subjects in the 6- and 3-mg dose groups relative to 7% in the placebo group.[172] Additionally, 32% and 19% of subjects in the 6- and 3-mg dose groups experience at least 1 adverse event leading to temporary interruption or discontinuation of study drug relative to 4% of subjects administered placebo. Incidence of diarrhea were also higher in treated subjects (36% and 41% at the 6- and 3-mg dose levels, respectively) relative to placebo (22%), as were abdominal pain, nausea and abdominal distension.[172] In an exploratory, non–placebo-controlled, 12-week study, 67% (1 mg) and 74% (3 mg) of subjects showed an improvement in steatosis, 33% (1 mg), and 42% (3 mg) showed improvement in inflammation,

42% (1 mg) and 53% (3 mg) showed improvement in ballooning, and 25% (1 mg) and 42% (3 mg) showed improvement in fibrosis, as assessed by liver histology.[173] Overall, initial clinical results suggest FGF19 agonism may be a viable treatment for NASH, but as with all current NASH assets, more studies need to be conducted in humans to assess the side effect profile and tolerability of this mechanism for longer term chronic treatment.[174]

Conclusions

Novel therapies are being evaluated and developed for NASH at a rapid rate, with most agents targeting at least some aspect of metabolic dysfunction. While many of these established mechanisms are covered in this review, there are additional emerging areas under investigation for effects on hepatic metabolism including AMPK activation,[175,176] sirtuins,[177,178] and the gut microbiome.[179] It should be noted, however, that metformin, which activates AMPK, has not impacted histological endpoints in NAFLD or NASH despite showing improvements on HOMA-IR and liver function.[180] Disruption in the gut-liver barrier (aka "leaky gut") is common in NASH and may be an important link between the transition from simple steatosis to a more severe form of the disease.[181] Although NASH is well accepted as a "metabolic" disease, with insulin resistance, obesity, and hepatic steatosis as major risk factors predisposing to disease pathogenesis, there is heterogeneous susceptibility in the progression from NAFLD to NASH. Part of this variability may be explained by genetic predisposition as emerging human genetics has revealed several genetic risk variants for NAFLD or NASH, including mutations in PNPLA3 (148M), TM6SF2 (E167K), GCKR, and hydroxysteroid dehydrogenase 13 (reviewed in[182–186]). Emerging analysis of the liver transplant literature suggests that NAFLD, and even NASH, will recur in the new liver at a greater rate if underlying metabolic dysfunction is not "cured," providing support for the notion that NASH is a metabolically driven disease.[187,188] Indeed, lifestyle modification through diet and physical activity remains at the forefront of NASH management and has the potential to improve overall metabolic health and predisposing NASH risk factors. However, long-term compliance to lifestyle changes is a challenge for most patients, underscoring the urgent need for effective pharmacotherapies for patients with NASH.

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Conflicts of interest
The authors declare the following: William P. Esler and Kendra K. Bence are employees of Pfizer, Inc and may be shareholders of Pfizer, Inc.