Review

MOLECULAR METABOLISM

FGF19 and FGF21: In NASH we trust

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ABSTRACT

Objective: FGF19 and FGF21 have shown therapeutic promise since their discovery, attested by the fact there are at least 5 assets that activate the FGFR/KLB pathway and one FGF19 analog in clinical development.
Methods: We performed a detailed analyses of published preclinical and clinical data to offer insights into the mechanism of action, as well as PK/PD and efficacy data of the clinical assets.
Results: Scouring the literature, we offer mechanistic insights from preclinical data using rodents and non-human primates and pharmacodynamic data from clinical studies.
Conclusion: The basic and applied science around endocrine FGFs has evolved exponentially over the years with FGF19 and FGF21 analogs are now entering Phase 3 clinical research.

Keywords FGF21; FGF19; NASH; Metabolism; Drug development; Clinical trials

1. INTRODUCTION

Fibroblast growth factors 19 and 21 (FGF19 and FGF21) are novel endocrine messengers that regulate multiple aspects of energy homeostasis. The magnitude and pleotropic character of their beneficial actions on many, if not all, abnormalities of the metabolic syndrome in animals has led to extensive exploration of their biology and coordinated efforts to design novel FGF19/21-based analogs for therapeutic purposes. While initial attempts to develop such medicines were primarily focused on improving hyperglycemia in type 2 diabetes patients, the robust, consistent, and durable effects on lipid metabolism in human trials gradually transformed clinical emphasis for these factors toward their use for non-alcoholic steatohepatitis (NASH) and severe hypertriglyceridemia (SHTG). In this review, we will communicate an overview of FGF19 and FGF21 biology and the basic mechanisms of their action, which will be followed by in-depth analysis of the recent developments in FGF21/19-based analogs in the clinic.

2. ESSENTIALS OF FGF19 AND FGF21 BIOLOGY

FGF19 and FGF21 are atypical members of the FGF superfamily. As opposed to classical FGFs, these proteins have only weak or no heparin-binding affinity, allowing them to enter into general circulation and function in an endocrine manner to regulate various aspects of lipid and carbohydrate metabolism [1–5]. FGF19 and FGF21 were cloned almost simultaneously, in 1999 [6] and 2000 [7], respectively, and both factors have emerged as key metabolic regulators. Due to the robust pharmacology in the original experiments in mice [8,9], their corresponding pathways have become one of the most studied mechanisms of our time.

Circulating FGF21 is liver-derived [10], but it also expressed in a number of other tissues, such as pancreas, muscle, and adipose, where it is thought to be acting in an autocrine/paracrine manner [11–13]. FGF21 is shown to be significantly elevated upon food deprivation and feeding ketogenic diet in rodents [14,15] and prolonged fasting in humans [16,17], leading to the idea of FGF21 being a starvation or 'Atkins'-like hormone. In contrast, FGF19 and its mouse ortholog FGF15 [18] are gut-produced hormones with the highest expression in the ileum [11]. FGF19 is elevated in human plasma postprandially via activation of bile acids (BA)-farnesoid X receptor (FXR) axis [19] to repress the expression of the rate-limiting enzyme CYP7A1 in the liver that controls BA synthesis [20]. While genetic studies are indicative of an overlap in murine FGF15 and FGF19 functions, pharmacological experiments seem to suggest some functional divergence in actions between these two proteins in mice [21], perhaps due to the presence of an unpaired cysteine in FGF15 that is nonexistent in other endocrine FGFs [22]. This makes the FGF15 protein prone to dimerization, thus making it challenging to produce in a fully functional form.

Both FGF19 and FGF21 signal via FGF receptors that are widely expressed in the body. Soon after cloning, FGF19 was shown to bind FGFR4 but not the other FGF receptor isoforms [23]. It was later determined that direct engagement of FGFR4 by FGF19 is relatively inefficient [24], and for its full activity, this factor requires a transmembrane scaffold protein βKlotho (KLB) [4,25]. The presence of KLB also allows FGF19 to activate other FGF receptors, FGFR1-3, thus widening the tissue targeting for this hormone beyond liver, where FGFR4 is abundantly expressed [26]. While FGF21 was shown to

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Received September 29, 2020 • Revision received December 4, 2020 • Accepted December 18, 2020 • Available online 29 December 2020

https://doi.org/10.1016/j.molmet.2020.101152

activate FGFR1 and FGFR2 in 3T3-L1 adipocytes via an unknown co-factor [9], the mechanism and precise isoform specificity of FGFR engagement by FGF21 remained unclear until the strict KLB requirement for the action of this factor was uncovered [27–30]. Indeed, the cellular expression of KLB allows FGF21 to engage FGFR1, 2, and 3, but not FGFR4 [31] even though FGF21 binds this receptor *in vitro* in the presence of KLB [27,28]. Furthermore, as this co-factor is selectively expressed in adipose, liver, and pancreas tissue [11], KLB defines the profile of tissue targeting for FGF19 and FGF21 in animals. In humans, KLB expression is also apparent in some other tissues, whereas FGFR1 expression is ubiquitous [32], unlike what has been reported in rodents [11].

The C-terminal tails of FGF19 and FGF21 underlie the ability of these hormones to bind KLB, while N-termini of either factor determine their FGF receptor specificity [33,34]. The recent crystallography and peptide-based studies mapped FGF/KLB interaction interface at a single amino acid resolution [35,36]. Since the tissue sensitivity to FGF19 and FGF21 depends on their KLB-binding affinity and the expression levels of this co-factor [37–40], engineering of FGF analogs with superior potency compared to native forms for therapeutic purposes becomes feasible. Likewise, FGF21 and FGF19 variants with altered receptor signaling specificities can be developed via modifications in their N-terminal structures [41,42].

Thus, the mechanisms and the spectrum of FGF receptors by which FGF19 and FGF21 propagate their signals are similar (Figure 1), explaining why the outcomes of the pharmacological interventions with these factors are largely analogous in cell cultures and animals [24]. Yet, the ability of FGF19 to induce liver cancers in mice via FGFR4 activation [31,43] is of significant concern, which does not appear to be the case for FGF21-based analogs [44–46]. Several attempts were made to engineer out the mitogenic component in native FGF19, and proliferation-free variants currently exist [47,48]. One of them, NGM282 also referred to as Aldafermin, is a full FGFR4 agonist, but due to its inability to activate STAT3 pathway, it lacks tumor-promoting activity in mice [42,49]. Aldafermin is now being pursued in Phase 2 clinical development for NASH and primary sclerosing cholangitis [50]. Another potential issue with FGF19-based analogs is an undesirable LDLc elevation in human patients even though it can be mitigated by statin co-administration [51]. The rise in LDLc with Aldafermin in humans appears to be an on-target effect, due to activation of FGFR4-Cyp7A1-cholesterol/BA synthesis axis, which is an intrinsic aspect of FGF19 biology. In contrast to FGF19, FGF21 analogs, consistently lower LDLc in non-human primates (NHPs) and clinical trials [52–54].

FGF21 is renally cleared, as evidenced by its accumulation in subjects with kidney disease [55], and when delivered via either intravenous (IV) or subcutaneous (SC) routes in mice or NHP, its plasma half-life is between 0.5-0.6 and 2–4 h, respectively [56]. Circulating FGF21 can also be proteolytically cleaved at multiple sites, and the specific truncation of the last 10 amino acids by fibroblast activation protein (FAP), the endopeptidase belonging to the DPPIV family, essentially renders the protein functionally inactive [57–59]. While FAP may represent a novel target in metabolic research, questions remain about plasma/tissue levels of native FGF21 required to elicit therapeutically meaningful responses in rodents and humans [60]. Given its size, FGF19 must also have a short-half life in circulation, which is indirectly evidenced by once daily dosing adapted for the Aldafermin analog [61]. No proteolytic cleavage has been reported for FGF19 so far [42]; however, ongoing clinical studies with Aldafermin could offer insights into potential metabolites.

Physiologically, FGF19 is considered to be a key factor that modulates BA/cholesterol synthesis, whereas FGF21 is believed to be a major regulator of glucose and lipid homeostasis. Nevertheless, the

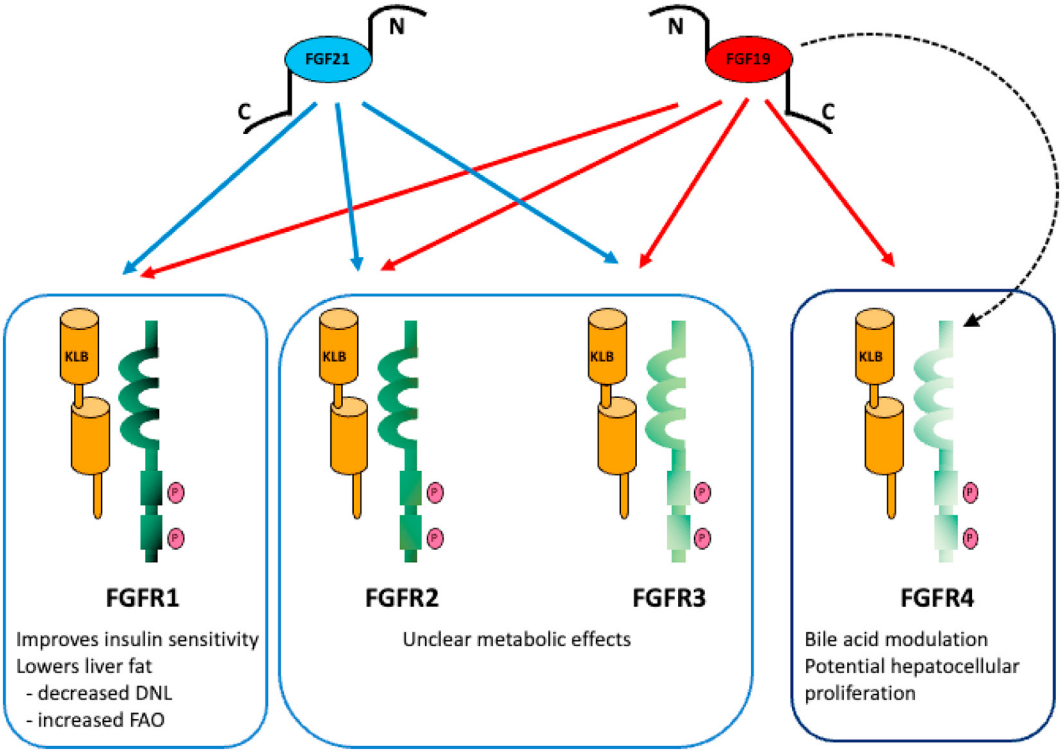

Figure 1: Human FGF Receptor/KLB selectivity for FGF21 and FGF19.

phenotypes of FGF19 and FGF21 transgenic animals are nearly identical [9,62]. Both mouse strains are lean and diet-induced obesity (DIO)-protected and show reduced plasma glucose, lipids, insulin, glucagon, leptin, lower liver fat content, and increased energy utilization. Nearly identical effects at the whole-body level are seen with administration of FGF19 and FGF21 proteins in animals, and mechanistically similar sets of genes are regulated by both factors in the periphery and in the brain [8,9,63,64]. Furthermore, FGF21 may also function as a regulator of cholesterol/bile acid synthesis in mice, albeit at somewhat reduced potency, when compared to FGF19 [65]. Whether these parallels are related to cross-species pharmacological artifacts allowing human FGF21 protein to function via mouse KLB [24] as opposed to human KLB/FGFR complex [31], and the fact that human FGF19 being more potent in mice than its mouse FGF15 ortholog [21], remain to be determined. Yet, the purported physiological roles for these factors seem to decouple from properly predicting activities of FGF19 and FGF21 in pharmacological settings [66].

Since native FGF21 showed promise in preclinical species to treat metabolic disease [9], harnessing its full therapeutic benefits has become a priority for industry research. In animals, FGF21 is one of the most robust acute insulin sensitizers [67] with FGF21-dependent insulin lowering apparent in rodents within 15 min after a single dose [68], and it is thought to primarily act via signaling in white and brown adipose tissue [12,67,69]. Multiple reports have also demonstrated FGF21-dependent lowering of body weight (BW), fat mass, blood glucose, insulin, lipid levels, and hepatic steatosis as well as the increases in total energy expenditure (EE) and physical activity in DIO mice [63,67,70–72]. The decrease in hepatic triglycerides (TGs) occurs through inhibition of nuclear sterol regulatory element-binding protein-1 (SREBP1) and reduced expression of genes involved in fatty acid and TG synthesis [64], while plasma TGs in mice are lowered by reduced very low-density lipoprotein (VLDL) secretion and modulation of fatty acid uptake in white adipose tissue [73]. In rats, ICV administration of FGF21 improves hepatic insulin sensitivity, increases food intake and EE without causing weight loss [74]. Interestingly, ob/ob, db/db mice and Zucker rats have little to no change in BW upon FGF21 administration [24,72,75,76], suggesting the requirement of a functional leptin pathway for its weight-regulating function. Nevertheless, the glycemic control upon FGF21 administration is fully retained in these leptin pathway-deficient rodent models [24,72,75,76], indicative of a molecular partitioning of FGF21 mechanism for glucose and weight control in animals.

3. TARGET TISSUES: ADIPOSE, LIVER, AND CENTRAL NERVOUS SYSTEM (CNS)

Preclinical data in rodents have indicated that white adipose tissue plays an important role in FGF21 and FGF19 pharmacology. KLB expression in adipose tissue is required for the acute insulin sensitizing effects of FGF21 [29], while adipose-specific FGFR1 KO [68,77] and lipodystrophic mice are largely refractory to the beneficial effects of pharmacological FGF21 administration [78]. Adiponectin, an adipose tissue-produced hormone, has been implicated in mediating the glycemic and insulin-sensitizing effects of FGF21 in mice, and proposed to act on the liver, to mediate systemic effects of FGF21 [79]. Of note, circulating adiponectin is consistently elevated in conditions of FGFR/KLB pathway activation in all species reported so far and is likely to represent a clinical biomarker for target engagement of pathway activation.

Hepatocytes that express high levels of FGFRs and KLB [11] are direct targets for FGF19 and FGF21 actions [80,81], which underlies the potential for these factors to treat fatty liver disease. Furthermore, there is emerging evidence that hepatic stellate cells (HSCs) that drive fibrogenesis in the liver can also be modulated by these hormones [82]. In mouse models, FGF21 attenuates dimethylnitrosamine (DMN)-induced hepatic fibrogenesis, directly signaling in activated stellate cells by downregulating the expression of transforming growth factor beta (TGF-β) and nuclear translocation of nuclear factor kappa beta (NF-κB) and causing apoptosis of these cells [83]. Importantly, the anti-fibrotic effects with FGF21-based analog has been recently reported in a clinical study [84]. The effects of FGF19 on HSC-mediated fibrogenesis are less clear. While FGF15 null mice develop attenuated liver fibrosis in the CCl4-induced fibrosis model, pharmacological administration of FGF15 and FGF19 do not act as direct profibrotic mediators or mitogens to HSCs or human LX2 cells [85]. Treatment of NASH subjects with Aldafermin showed beneficial effects on fibrosis [50,86].

SNPs in and around the FGF21 gene have been reported to be associated with enhanced preference for carbohydrates, raising the question of whether altered FGF21 levels can impact macronutrient choice [87,88]. Several preclinical studies have demonstrated that FGF21 may elicit some of its effects through its action in the CNS [89]. KLB ablation with a calcium/calmodulin-dependent protein kinase II a (Camk2a)-Cre line that expresses Cre recombinase in the forebrain and hindbrain renders mice refractory to the effects of FGF21 on body weight [90]. Moreover, pharmacological administration of a long-acting FGF21 analog in these animals did not cause weight loss compared to littermates, further implicating a role of the CNS in mediating weight loss [91]. Circulating FGF21 levels are robustly increased by diets that are high in carbohydrates but low in protein, mediated by the transcription factor carbohydrate Responsive-element binding protein (ChREBP), which is potently activated by fructose [92,93]. Furthermore, genetic or pharmacological modulations of FGF21 or its co-receptor KLB impacted carbohydrate preference in mice and NHPs [32,94]. These data indicate the existence of a novel feedforward loop in which ingestion of sweet food/water increases FGF21, allowing this hormone in the CNS to suppress further consumption of sweets, with a concomitant reduction in dopamine levels in the reward center of the brain [95]. Importantly, mice lacking FGF21 signaling in the CNS are unable to shift macronutrient preference, resulting in increased protein intake in response to dietary protein restriction [93].

Consumption of fructose by healthy subjects causes a robust and rapid elevation of FGF21 levels [96]. Following up on the sweet preference in rodents, FGF21 was demonstrated to decrease carbohydrate intake in rodents via signaling to glutamatergic neurons in the ventromedial hypothalamus and markedly enhanced glucose sensitivity of KLB neurons in the VMH [97]. In a recent study conducted in obese subjects, a single dose of the bispecific anti-FGFR1/KLB agonist antibody, BFKB8488A showed a trend toward reduction in preference for sweet taste and carbohydrate intake [98]. Longer duration studies with a larger sample size will be needed to demonstrate whether this effect holds up and provide mechanistic insights. Since carbohydrates, and in particular fructose, is known to cause hepatic steatosis, it is tempting to speculate whether the beneficial effects of pharmacological administration of FGF21 on hepatic steatosis is mediated at least in part, via decreased carbohydrate ingestion. In contrast, the role of CNS in propagation of pharmacological effect of FGF19 is less studied. However, ICV-delivered protein reduces food intake, glucose levels, and body weight in rodents [99,100].

4. PHARMACOLOGY IN NHP

Native FGF21 [56] and a Lilly-designed FGF21 variant LY2405319 [101] were both tested in diabetic Rhesus monkeys. Native FGF21 caused a significant decrease in fasting plasma glucose, fructosamine,

TGs, insulin, and glucagon, without incidence of hypoglycemia. Also, significant improvements in lipoprotein profiles, including lowering low-density lipoprotein cholesterol (LDLc) and increasing high-density lipoprotein cholesterol (HDLc), and beneficial changes in the circulating levels of several cardiovascular risk markers/factors were observed. A small but significant weight loss was noted, while estimated caloric intake was variable over the course of this study. When tested at much higher doses (100×), LY2405319 produced similar pharmacodynamic effects [101], yet the onset of the effects was much faster than reported in [56]. Increased adiponectin and reduced leptin levels in this study were suggestive of direct FGF21 action on adipose tissue in NHPs, consistent with the observations in rodents. Food consumption in this study was decreased by more than 50%, suggestive of decreased caloric intake being the primary mechanism, leading to weight loss in higher species [101].

Subsequently, Amgen dosed wild-type FGF21 and a long-acting FGF21 molecule Fc-FGF21-RG in obese monkeys, with both molecules showing decreases in body weight, glucose, insulin, cholesterol, and TG levels, and an improved glucose excursion during glucose tolerance test, yet Fc-FGF21-RG was pharmacologically superior [102]. In 2012, Amgen reported a design of a fully human monoclonal antibody, termed "mimAb1," that activated the FGFR1c/KLB, but did not activate FGFR2c and FGFR3c complexed with KLB. In obese cynomolgus monkeys, treated with mimAb1, FGF21-like beneficial effects on metabolism were observed. Such as decreases in body weight, plasma insulin, and TGs [77]. Although this molecule showed impressive efficacy, it is unclear whether this asset has been used in a clinical setting.

Pfizer tested a clinical candidate, PF-05231023, human immunoglobulin G (IgG) coupled to two modified FGF21 molecules with intact N- and C-termini [72] in spontaneously obese male cynomolgus monkeys [91]. Again, a profound decrease in BW was noted, driven almost entirely by decreased caloric intake [103]. Reduced food consumption and BW effects were apparent a few hours post the first dose, sustained throughout the dosing period, and returned to baseline during washout. The weight loss was accompanied by decrease in abdominal circumference and reductions in axial fat, without changes in bone mineral content even in the face of 10% weight loss [91]. This was one of the first studies to show that bone loss, which had been previously reported in FGF21-dosed mice with FGF21 [104], does not translate to primates with the use a long-acting FGF21 molecule consistent with the observations reported elsewhere [105]. Weight loss in humans leads to increased bone turnover, which is proposed to be due to a reduction in mechanical stress on the weight-bearing skeleton, particularly the hip and spine, resulting in changes in circulatory biomarkers [106,107]. Calorie restriction studies in humans with a very low-calorie diet (VLCD) causes bone loss that is often proportional to the amount of weight lost [108]. Longer duration, placebo-controlled studies with a larger population will likely establish the FGF21 and bone axis in humans, which should include bone mineral density measurements, in addition to circulating markers of bone turnover. PF-05231023 also caused a robust decrease in circulating TGs of 80% placebo-adjusted at the highest dose, an effect that was maxed out and sustained from day 8 until the end of treatment. Consistent with observations in humans [52], there was also an apparent elevation of serum ketone bodies at the highest dose.

A PEGylated FGF21 variant, B1344, was administered in obese male cynomolgus monkeys, the first study to test activators of the FGF21 pathway in NHPs with established fatty liver [109]. Consistent with other FGF21 molecules, B1344 caused a profound reduction in body weight, TG, VLDL, and food intake, contributing to weight loss. Consistent with the data reported for the PFE molecule, there were no changes in bone mineral density measured by dual-energy X-ray absorptiometry (DEXA) despite the observed 10% weight loss. B1344 also induced a 40% decline of liver fat content (LFC) as measured by magnetic resonance imaging-proton density fat fraction (MRI-PDFF). Histology data showed improvement of steatosis in NAS, with no changes in hepatocyte ballooning and inflammation. B1344 decreased fibrosis, myeloperoxidase (MPO), which is a neutrophil marker, and epidermal growth factor (EGF)-like module-containing mucin-like hormone receptor-like 1, also known as F4/80, a macrophage marker, with staining representing a decreased inflammation tone. Longer duration studies may be needed for histological changes to be observed that are consistent with decreased immune infiltrate in the liver [109].

89BIO-100, a novel site-specific glycoPEGylated analog of fibroblast growth factor 21 (FGF21) molecule, was administered to spontaneously diabetic cynomolgus monkeys subcutaneously once weekly for 8 weeks. BIO89-100 caused a significant reduction of body weight, TG, and LDLc. Consistent with reports with other FGF21 analogs, BIO89-100 increased adiponectin levels by almost two-fold. Moreover, in an oral glucose tolerance test (OGTT), 89BIO-100 improved glucose excursion during OGTT suggesting an improved glucose tolerance in these animals. The improvement in OGTT was in the face of weight loss, which could have contributed at least in part to improved glucose tolerance [110].

A humanized anti-FGFR1/KLB agonist antibody BFKB8488A induced dose-dependent weight loss in obese cynomolgus monkeys. DEXA analyses demonstrated that the weight loss was caused to a large extent, by a decrease in fat mass, with minimal decrease in lean mass, and was due to reduced food intake, which was almost completely suppressed in the highest dose group. Consistent with decrease in food intake, serum levels of β-hydroxybutyrate (BHOB) and non-esterified fatty acids (NEFAs) were elevated, as well as a dose-dependent increase in high molecular weight adiponectin.

Key endpoints upon pharmacological administration of all FGF21 analogs in NHP are tabulated in Table 1. Data in different manuscripts have been reported in absolute or relative numbers.

5. FGF21 AND FGF19 ANALOGS IN CLINICAL DEVELOPMENT

The first FGF21-based molecule to enter clinical development was a modified FGF21 variant, LY2405319 (LY), designed for improved biopharmaceutical properties and yeast expression [111]. Consistent with what was reported preclinically for this molecule and mechanism, I patients with type 2 diabetes, LY caused a modest but significant weight loss and favorable changes in lipid profiles as demonstrated by robust decreases in TGs and LDLc, increases in HDLc and adiponectin, and reductions in apolipoproteins A2, B, and C-III levels [52]. LY also increased circulating levels of β-hydroxybutyrate suggestive of elevation in fatty acid oxidation. Significant lowering of fasting insulin indicative of improvements in insulin sensitivity was also noted, yet no significant decrease in fasting glucose was observed in this study. The latter is contrary to the data in rodents, where FGF21 and LY consistently lowered blood glucose [9,111] and could be attributed to a number of reasons, including but not limited to insufficient activity in humans and/or the short, four-week duration of this study. Indeed, the onset and efficacy of glucose reductions with FGF21-based therapies in monkeys are robustly improved at higher doses [101,112], and KLB-binding improved FGF21 analog, Fc-FGF21-RGE or AKR-001 (Efruxifermin) [113], showing robust reductions in HbA1c levels upon 16 weeks of dosing in humans [84].

Table 1 — Comparative efficacy of different FGF21 assets in non-human primates.

	Lilly Native FGF21	Lilly LY2405319	Pfizer PF-05231023	Novo	Amgen	Amgen Fc-GF21 (RG)	Amgen Fc-FGF21	Amgen mimAb	B1344	89BIO-100	Genentech BFKB8488A
Modality	Native FGF21	FGF21 variant	CovX-FGF21	Met-FGF21	rhFG21	Fc-FGF21 (RG)	Fc-FGF21	Bispecific Ab	PEGylated FGF21	Glyco-PEGylated FGF21	FGFR1/KLB Agonist Ab
Species	Rhesus	Rhesus	Male cyno	Rhesus	Rhesus	Rhesus Cynomolgus	Cynomolgus	Cynomolgus	Cynomolgus	Cynomolgus	Cynomolgus
Body weight	− 1–4% from baseline	− 2.8 kG	− 11%	− 17.6%	Modest decrease	−0.8 kg and -2 kg	−1 kg	− 15%	−12%	−12%	−15%
Food intake	Not significant decrease	− 75%	− 50%	No change	NR	No change −80%	No change, −80%	"Slightly decreased"	Decrease	NR	−80%
Triglycerides	−69%	− 90%	−78%	- 50%	Significant decrease	Significant decrease NR	− 50%	− 50%	− 50%	−70%	NR
Fasting glucose	−50 mg/dL	− 64%	No change	No change	No change in OGTT	Significant decrease in OGTT NR	No change	No change	NR	Improved OGTT	NR
Fasting insulin	−100 uU/mL	- 60%	No change	− 50%	Decrease in OGTT	Significant decrease in OGTT NR	− 50%	− 50%	NR	NR	NR
Adiponectin	+1.8 fold	+4-fold	+3-fold	+2-fold	NR	NR and NR	+2–3 fold	No change	+50%	+2 fold	+3.5 fold
LDL-C	−28%	−38%	No change	No change	NR	Decreased	NR	Not reported	NR	−40%	NR
VLDL-C	−160 mmol/L	− 90%	− 80%	− 75%		NR			−50%	NR	
HDL-C	+79%	+50%	+40%	Not reported	NR	NR	NR	Not reported	NR	NR	NR

It is important to note that the kinetics and magnitude of TG lowering is superior to that of other metabolic endpoints, and this feature of FGF21 biology is consistent across species and modalities tested. LY caused a significant decrease in TGs as early as Day 3 of dosing with the effect reaching 50% maximal reduction by Day 7 and remaining at this level for the duration of the study [52]. These data suggest partitioning of FGF21 activity across individual metabolic readouts in humans, and that the TG lowering mechanism is likely distinct from that of other pharmacological endpoints. In rodents, FGF21 augments lipoprotein catabolism in white and brown adipose tissues, leading to lower circulating TGs [73]. While the clinical development of LY2405319 compound was discontinued likely due to the absence of a robust glycemic effect, this study was the first to demonstrate the therapeutic utility of the FGF21 pathway in humans and set the stage for other molecules in this class to be explored for drug development purposes.

The second clinical experience with the FGF21 pathway comes from a Pfizer report on a long-acting FGF21 variant, PF-05231023 that allows once or twice-weekly dosing [91]. In type 2 diabetes subjects, PF-05231023 caused a significant decrease in BW apparent at Day 8 that continued to decrease over the period of dosing, amounting to a 4.2% reduction at the end of administration on day 28. Consistent with data on Lilly molecule PF-05231023 potently improved lipid profiles, but there was little to no change in insulin, and changes in blood glucose observed were not significant. This finding was surprising because a significant decrease in insulin levels was noted earlier with LY2405319, and a placebo-corrected 4.2% weight loss was expected to induce improvements in glycemic/insulin parameters. Furthermore, PF-05231023 molecules showed unfavorable changes in bone biomarkers, yet no orthostatic changes were observed in this study. Of note, a VLCD leads to BW loss in humans and is typically accompanied with changes in bone biomarkers similar in magnitude to those observed with the PFE molecule. This effect is likely secondary to weight reduction [91].

The follow-up study with PF-05231023 was conducted in obese hypertriglyceridemic subjects [53]. Consistent with the previous report and other studies, PF-05231023 caused beneficial changes in lipid profiles. Yet, in a stark contrast to the previous findings, no weight loss with PF-05231023 dosing was observed in this study, although bone biomarkers trended in a similar direction as in [91]. Even more surprising, PF-05231023 in this experiment caused increases in blood pressure and heart rate even though no changes in vital signs at the same doses were noted previously [91]. Given these uncertainties on the efficacy and safety profiles of PF-05231023, the development of this molecule has likely halted. Pfizer communicated on another glycoengineered long-acting FGF21 variant, PF-06645849, with improved pharmacokinetic properties to enable weekly to twice monthly subcutaneous dosing. This molecule demonstrated robust glucose lowering and weight loss in DIO mice [114], but it had not been progressed to the clinic as of July 2020.

BMS is developing a PEGylated FGF21 analog (BMS-986036) to treat obese type 2 diabetes subjects at risk for NASH. Safety/tolerability and HbA1c were the primary endpoints in a randomized, double-blinded 12-week study with BMS-986036. This molecule was well tolerated in general, and most adverse events (AEs) were mild and not dose-dependent. The most common AE was diarrhea, which was more apparent in the active arms compared to the placebo group. Although there were two subjects with serious AEs, none of these events were considered drug-related. There was no change in HbA1c at completion of treatment, although there was a modest trend of reduced BW, insulin, and homeostasis model assessment-estimated insulin resistance (HOMA-IR) in the 20 mg QD daily dose. Consistent with other

FGF21 molecules, BMS caused beneficial changes in lipid profiles [115]. Lowering of fibrotic biomarkers, such as PRO-C3, PAI-1, and YKL-40, was also observed as well as favorable but modest changes in liver enzymes [115] Taken together, this experiment led the ground work for their subsequent study described below.

In a Phase 2a study conducted in obese/overweight subjects with BMIs of at least 25 kg/m^2 and patients with biopsy-confirmed NASH, BMS-986036 (or Pegbelfermin) caused a significant reduction of liver fat measured by MRI-PDFF [116]. At a 10 mg once daily dose (QD), a 6.8% decrease in absolute liver fat from baseline was observed, while at the 20 mg once a week dose (QW), this effect was 5.2%. At 16 weeks, the relative change in hepatic fat fraction was 56% lower than baseline in the 10 mg QD group, and liver enzymes were decreased. In the 20 mg QW group, there was a modest decrease of liver stiffness, yet this change was not observed in the 10 mg QD cohort. This could be attributed, at least in part, to the small sample size and/or insufficient study duration. There was a robust and significant reduction of PRO-C3 at both dose groups compared to placebo. As of July 2020, there were two active studies listed in subjects with NASH and cirrhosis and Stage 3 liver fibrosis, respectively [117]. In a 12-week phase 2 study, QD and QW administration of Pegbelfermin in patients with obesity and type 2 diabetes caused significant increase in HDLc and decrease in TGs. However, there were no statistically significant changes observed in HbA1c, weight, fasting insulin, C-peptide, and HOMA-IR levels [115]. Taken together, these favorable results in the NASH subjects warrants further investigation of this asset in the clinic.

The fourth molecule being clinically evaluated is Fc-FGF21-RGE, or AKR-001 or Efruxifermin (EFX) [84]. In the Ph 2a study, EFX was administered for 16 weeks in patients with NASH. The treatment with EFX was generally reported to be well tolerated, yet the frequency of drug-related a diarrhea and nausea was approximately 30+%. Out of 40 treatment responders who had end-of-treatment biopsies, 48% achieved at least a one-stage improvement in fibrosis without worsening of NAFLD activity score (NAS), and 28% achieved at least a two-stage improvement in fibrosis. In addition, 48% of responders achieved NASH resolution with no worsening of fibrosis. Improvements in dyslipidemia, weight loss, and 60−70% reductions in liver fat content were also observed across all dose groups. Of interest, AKR-001 significantly reduced HbA1c by approximately 0.5% in this study contrary to the earlier hypotheses that the ability of FGF21 to regulate glycemia in rodents and NHPs may not be clinically translatable. More likely, studies longer than 28 days [52] and time-action/potency-optimized molecules are needed to reveal this effect in humans. In addition, EFX decreased BW of up to 3.7 kg in the 70 mg group. EFX improved circulating lipid profiles by causing a significant decrease of TG of 43%, increase of HDL by 40%, and decrease of non-HDL cholesterol by 15%. Changes in TG, HDLc, and non-HDL were similar between the 50 and 70 mg groups, that suggesting the effects plateaued at the 50 mg dose. EFX also caused small but significant percentage-wise decreases in HbA1c of −0.1, −0.4, and −0.5 at 28, 50, and 70 mg, respectively, with placebo at +0.1%. No changes in LDL were observed at higher doses, although it was significantly decreased by 14% in the 28 mg group. There were no treatment-related effects on blood pressure, heart rate or bone mineral density. Interestingly, in a previous study [118], EFX decreased specific apolipoproteins secreted by the liver as components of VLDL (apoB) apoC-2, and apoC-3 and increased apoA-1, which are indicative of AKR-001 potentially modulating protein expression in human hepatocytes. It is therefore possible that EFX elicits these changes due to a direct effect on hepatocytes. However, additional studies are required to firmly establish this. The effect of EFX on circulating Apo C-2 and Angptl-4 levels are novel and unlike what has been reported for other clinical assets targeting this pathway. Since EFX is balanced across the FGFR1c, FGFR2c, and FGFR3c complexed with KLB, additional data will be the key to understand the potential roles of FGFR2c and FGFR3c toward efficacy.

The finding that AKR-001 increases glucagon is both novel and intriguing in terms of FGF21 biology in humans. There are reports showing that native glucagon increases plasma FGF21 levels in human subjects, and a synthetic glucagon receptor agonist (IUB288) upregulates FGF21 expression in isolated primary hepatocytes from wild-type, but not glucagon receptor−null, mice [119]. This report has led to the suggestion that glucagon controls glucose levels, energy, and lipid metabolism, at least in part, via FGF21-dependent pathways. Evidence to the contrary is lacking in that there are fewer reports measuring glucagon action upon pharmacological administration of FGF21. In mice treated with FGF21, glucagon levels are unchanged, but NHPs treated with FGF21 analogs showed decreased glucagon. Regardless of the mechanism, it is plausible that elevations of ketone bodies reported by the Lilly molecule and AKR-001 in humans is a consequence of elevated glucagon. Additional studies are needed to parse out this effect in humans with specific context of FGF21 pharmacology.

On September 14, 2020, 89Bio disclosed their Phase1b/2a topline results for BIO89-100, which is a glycoPEGylated FGF21 variant, 89BIO-100. This molecule carries mutations in positions 173 and 176 with the glycoPEG attached to position 173, which extends the half-life of this molecule to 55−100 h based on a SAD study. 89BIO-100 has low nanomolar potency toward FGFR1c, FGFR2c and FGFR3c, which is similar to the activity of native FGF21, and does not signal via FGFR4. BIO89-100 was administered in 5 different doses for 12 weeks in subjects with biopsy-proven F1-3 NAS score in subjects with pooled BMI of 34.8 and 40% pooled type 2 diabetes. This variant was dosed QW at 3, 9, 18, and 27 mg and Q2W at 18 and 36 mg. Relative reduction of liver fat from baseline at week 13 was up to 60% for the 27 mg QW and 50% for the 36 mg Q2W dose. In addition, the proportion of subjects with $\geq$30% relative reductions in liver fat were 86% for the 27 mg QW and 88% for the 36 mg Q2W group. In addition, BIO89-100 caused a significant reduction of TG, alanine aminotransferase (ALT), aspartate aminotransferase (AST), non-HDLc, LDLc, and ProC3 and increase in HDLc, which appeared to be inversely correlated with increased dose. BIO-89 decreased insulin, without changes in HbA1c and consistent with this pathway, significantly increased adiponectin levels. No meaningful changes in body weight were observed except in the 27 mg QW cohort that showed a significant reduction compared to placebo treatment. BIO89-100 had a favorable safety profile. Remarkably, mild gastrointestinal (GI)-related adverse events, such as diarrhea and nausea, were observed at the frequency which was even lower than in the placebo group. No tremor or changes in blood pressure and heart rate were reported. Fifteen point nine percent of subjects in the pooled BIO89-100 group reported mild increase in appetite, but this effect did not appear to be dose-dependent [120].

While all the above-described assets have been designed to be cleavage-resistant and long-acting FGF21 analogs, Genentech and NGM reported two mAbs that activate the KLB/FGR1c receptor/co-receptor system. NGM313 is a humanized, monoclonal antibody directed to KLB testing a in Phase 1b study in insulin-resistant subjects with NAFLD that were administered as a single dose of NGM313 240 mg SC or pioglitazone 45 mg QD for 36 days [121]. The primary objectives were changes in insulin sensitivity from baseline to Day 29 and LFC from baseline to Day 36. NGM313 caused a significant decrease in HOMA-IR, HbA1c, and fasting glucose on day 28 compared

Table 2 — Efficacy of different FGF21 analogs and modalities in humans.

	Lilly LY2405319	Pfizer PF 05231023	Pfizer PF-05231023	Akero (AMG-876) AKR-001	BMS BMS-986036	Genentech BFKB8488A	MSD MK-3655 (NGM313)	89Bio 89BIO-100
Modality	FGF21 variant	CovX-FGF21	CovX-FGF21	Fc-FGF21	PEG-FGF21	Bispecific Ab	mAb	glycoPEG-FGF21
Subjects	Obese, T2D	Obese, T2D	Obese, hypertriglyceridemic 6	NASH (BALANCED Ph 2a)	NASH	NAFLD	Insulin resistant, NAFLD	NASH or phenotypic NASH
Doses (frequency)	3, 10, 20 mg (QD) SC	5, 25, 100, 140 mg (Q2W) IV	25, 50, 100, 150 mg QW IV	28, 50, 70 mg (QW) SC	10 mg (QD), 20 mg (QW) SC	50, 75, 100, 130 mg Q2W, 250 mg QM SC	240 mg, single dose SC	3, 9, 18, 27 mg QW 18 and 36 mg Q2W
Study length	4 weeks	4 weeks	4 weeks	16 weeks	16 weeks	12 weeks	36 days	12 weeks
Body weight	- ∼1.8% Pbo adjusted	− 4.2% (2x/wk)	No change (1x/wk)	−0.3, −2.3, −3.7 Kg Pbo +0.1	"no substantial changes"	NR	+1.2 kG from baseline D28	No change, and significant decrease in 27 mg QW
Triglycerides	− 44%	− 50%	−43.3% 150 mg	- 37, −45, −43 Pbo +8	−0.8 Pbo, −10.8 10 mg, −8.8 20 mg	−35% 250 mg QM −40% 130 mg Q2W	- 68.3 mg/dL from baseline D28	−28% from baseline 27 mg QW and 18 mg Q2W
Fasting glucose	− 4%	Numerical decrease	−0.11 mg/dL 150 mg −7.7 mg/dL Pbo	- 12−22%	NR	NR	∼5 mg/dL From baseline D28	NR
Fasting insulin	− 40% change from baseline	- 4%	−1.6 uU/mL 150 mg −4.4 uU/mL Pbo	"upto - 54%, (data not shown)"	NR	−55% + 21 (−55% at poorly tolerated doses)	Significant decrease	−6.9% 27 mg QW, −34.5% Q2W
HOMA-IR	Not reported	Not reported	NR	Not reported	NR	NR	- 2.6 from baseline D28	NR
HbA1c	Not reported	Not reported	NR	−0.1, −0.4, −0.5 Pbo +0.1	NR	Not reported	- 0.14 from baseline D28	−0.3 27 mgQW 0.5 in 36 mg Q2W
Adiponectin	+80% change from baseline	+60%	+3272 ng/mL 150 mg −223 ng/mL Pbo	+2−3 fold	−3.5% Pbo, 15.3% 10 mg, 15.7% 20 mg	+77% 250 mg QM +67% 130 mg Q2W	Significant increase	60.9% 27 MG QW, 24.1% 36 MG Q2W
LDL-C	− 20.2% LS Mean	− 30%	−4.8% 100 mg Pbo-corrected	−14, 0, −3 Pbo +1	- 14 mg/dL 10 mg QD	- 37% + 19%	- 15.8 mg/dL from baseline D28	−16% 27 mg QW −4% 36 mg Q2W
HDL-C	+19.5% LS Mean	+ ∼20%	+28.6% 150 mg Pbo-corrected	+32, +40, +40% Pbo 0	−0.8 Pbo, 5.9 10 mg, 5.2 20 mg mg/dL	+22% 250 mg QM +23% 130 mg Q2W	+7.4 mg/dL from baseline D28	+3% 27 mg QW +10% 36 mg Q2W
Liver fat MRI-PDFF	Not measured	Not measured	NR	NR	−1.3% Pbo −6.8% 10 mg, −5.2% 20 mg Absolute	−37% 250 mg QM −58% 130 mg Q2W RR	−37% RR D36	−60% 27 mg QW −50% 36 mg Q2W
Pro C3	NR	NR	NR	NR	2% Pbo, −33% 10 mg, −19% 20 mg	−12.5% 250 mg QM −37% 130 mg Q2W	- 14.3 from baseline D28	−27.7% 27 mg QW −12.8% 36 mg Q2W

to baseline, suggesting an improved insulin sensitivity. In a step hyperinsulinemic euglycemic clamp conducted in these subjects on day 29, NGM313 significantly increased the glucose disposal rate, the ratio of glucose disposal rate (GDR) and insulin (M/I), glucose metabolic clearance rate (MCR), and insulin sensitivity index (SI clamp, calculated from 2-step clamp). NGM313 significantly inhibited endogenous glucose production during clamp on Day 29 during the low-dose insulin infusion part of the clamp procedure. Whether this is due to a direct effect of NGM313 on the liver or secondary to improved insulin sensitivity in other peripheral tissues remains to be determined. NGM313 also caused a significant decrease in LFC from baseline on Days 23 and 36 by 30% and 37%, respectively. This study did not include a placebo arm, and hence, all data were reported as change from baseline. Consistent with previous reports, NGM313 caused a significant decrease of TGs and LDLc, and increase of HDLc on Day 28. Moreover, NGM313 decreased ALT, AST, and N-terminal propeptide of type III collagen (Pro-C3) by 14% on Day 28 compared to baseline. Pro-C3 is indicative of fibrogenic activity, increases with fibrosis stage, and is independently associated with advanced fibrosis in patients with NAFLD [122]. A significant increase in body weight by 1.2 kG on Day 28 was noted in subjects treated with NGM313. There were no significant changes in blood pressure, bone mineral density, or bone turnover markers. In January 2019, Merck and Co., Inc., Kenilworth, NJ, USA exercised its option to license NGM313. With the exercise of this one-time option, which was triggered by NGM's completion of the proof-of-concept clinical study of NGM313 described above, Merck and Co., Inc., Kenilworth, NJ, USA gained exclusive worldwide rights to develop, manufacture and commercialize NGM313, now renamed MK-3655, and related compounds [123–125].

Genentech's BFKB8488A is a bispecific agonist antibody that binds FGFR1 and KLB. In a first-in-human phase 1 trial, a single dose of BFKB8448A was administered subcutaneously in overweight/obese, healthy participants. BFKB8448A decreased body weight, fasting TG, LDL-c, plasma insulin, and fasting glucose, and increased HDL-c and adiponectin. Importantly, this is the first clinical study that reported appetite parameters, such as appetite sensations, and reported a significant decrease in % total kCal consumed compared to baseline, an effect that was driven to a large extent by decreased carbohydrate intake PMID: [98]. Importantly, in this study, although nausea and emesis were reported by subjects, the weight loss appeared to have preceded these AEs. However, given the sample size, it cannot be ruled out whether nausea led to decreased food intake, at least in part [98].

Genentech conducted a Phase 1b MAD study that tested 4 doses Q2W and 1 dose QM in NAFLD subjects for 12 weeks. This molecule demonstrated a favorable safety/tolerability profile, and significant increases in adiponectin and HDLc and decreases in TG and ProC3 were reported. There was a dose-dependent, relative reduction in hepatic fat fraction, measured by MRI-PDFF, of up to 38% at adequately tolerated doses ($\leq$100 mg Q2W) and up to 58% at the highest dose. GI effects at higher dose levels limited tolerability, with 100 mg Q2W and lower dose levels being adequately tolerated. Genentech appears to be progressing this molecule through clinical development in NASH and other related diseases [126].

In contrast to the substantial activity in the FGF21 space, the clinical efforts on FGF19 have been primarily led by NGM with its clinical asset NGM282, or Aldafermin, which is to our knowledge the only asset in clinical development for this mechanism. NGM282 is a first-in-class, engineered analog of the gut hormone FGF19. When this review was being written, NGM had completed studies in 4 cohorts. NGM282 was administered for 12 weeks in Cohorts 1–3 and 24 weeks in Cohort 4 with a total of almost 200 subjects combined in all dose groups. In Cohort 4 that used 1 mg of Aldafermin, 68% of Aldafermin patients achieved $\geq$5% absolute LFC reduction vs. 24% placebo, and 66% of Aldafermin patients achieved $\geq$30% relative LFC reduction vs. 29% placebo. Both of these changes were significant, at $p < 0.001$ and < 0.004, respectively. Thirty-eight percent of patients on Aldafermin *vs.* 18% on placebo showed fibrosis improvement of $>$1 stage without worsening of NASH and 24% Aldafermin vs 9% Pbo showed resolution of NASH without worsening of fibrosis. Notably, 22% subjects on Aldafermin achieved significant improvement in $\geq$1 stage fibrosis and NASH resolution at week 24. Of note, subjects on Aldafermin were administered statin therapy to mitigate LDL elevation, and hence, the potential confounding effect of statin's effect on fibrosis should be carefully monitored. Metadata analyses suggest statins are associated with reduced fibrosis in subjects with liver disease [127]. There were no changes in blood pressure or heart rate and no increase of pruritis reported in the Aldafermin study [128]. Mechanistically, Aldafermin engages all FGFR/KLB complexes which elicits the wide spectrum of responses observed with this pathway. Aldafermin reduces bile acid synthesis by inhibiting the conversion of cholesterol to bile acids. The latter is likely an underlying cause for an increase in serum cholesterol, and in particular, LDLc levels in humans. This mechanism was confirmed in a double-blind, randomized, placebo-controlled experiment in subjects with biopsy-proven NASH, in which Aldafermin decreased serum levels of C4, a surrogate marker of bile acid synthesis, which was strongly correlated with elevated LDLc. Changes in liver fat content associated with alterations in C4 and LDLc consistent with the purported FGF19 mechanism of action. Furthermore, such increase in LDLc aligns well with data showing that obeticholic acid, an agonist of the farnesoid X receptor and FGF19 secretagog, is also associated with elevated LDLc in the clinical studies. While NGM282 administration for two weeks increased LDLc at all doses tested, co-administration of rosuvastatin in these patients reduced total cholesterol and LDL concentration to the levels below baseline at the end of the study at 12 weeks [51]. All clinical data described above, is summarized in Table 2.

6. CONCLUDING REMARKS

As of September 2020, searches for FGF21 and FGF19 in PubMed pulled up more than 3000 scientific reports, an enormous progress since early days of endocrine FGFs' discovery 15 years ago. Along with basic advances in elucidating their physiology and mechanisms of action, preclinical pharmacology studies confirmed that these factors have favorable drug-like profiles in preclinical models of metabolic disease. Indeed, both FGF19 and FGF21 have shown robust, consistent, and durable benefits in successfully treating many, if not all, aspects of metabolic disease in rodents and primates. In parallel developments, clinical studies have emerged in the last decade revealing the promises and shortcomings of FGF therapies in humans. It remains to be seen whether current FGF19 and FGF21 assets can become registered products in the near future.

Despite intense ongoing research, the biology of both hormones has yet to be thoroughly established. Several outstanding questions still exist, such as why would nature create two separate hormones with nearly identical receptor recognition profiles and essentially overlapping pharmacologic signaling across multiple species? What is the need for a hormone that is robustly elevated upon food deprivation to cause weight loss in a pharmacological setting? Are carcinogenicity concerns with FGF19 in mice translatable to humans? What are the quantitative roles of adipose tissue and CNS in propagating signals of endocrine FGFs and the functions of these hormones in other KLB-

expressing tissues? Answers to these questions are not only relevant for expanding our understanding of the biology but also to inform on potential safety concerns.

The development path for FGF19 and FGF21 in the clinic was difficult at times. Since the native molecules are imperfect in time-action, solubility, and stability, protein engineering efforts were applied to correct their pharmaceutical deficiencies. Furthermore, while these hormones were initially positioned to treat hyperglycemia, early clinical studies showed little to no glucose lowering, leading to shear disappointment in the R&D community and de-prioritization of many clinical assets. Data eventually provide the required knowledge, and a robust magnitude of FGF19 and FGF21 effects to correct dyslipidemia in humans helped to overcome the development setbacks. NASH has recently emerged as an area of growing unmet medical need without approved therapies. Multiple strategies and research and development (R&D) programs are currently in place to develop effective treatments for this disease. Those include lifestyle interventions, bariatric surgery, the use of known anti-hyperglycemic agents, nuclear hormone agonists, metabolic enzyme inhibitors, and mitochondrial uncouplers [129]. Given the exceptional lipid-regulating efficacies of FGF19 and FGF21 in humans, these targets and their corresponding pathways are now investigated to tackle fatty liver and fibrosis using several FGF-based clinical analogs or mimetics. The definitive answers on the development prospects for these molecular entities are expected to arrive within the next couple of years

DISCLOSURES

S.T. is an employee and stockholder of Merck & Co., Inc., Kenilworth, NJ, USA
A.K. is an owner of AK Biotechnologies, LLC., Zionsville, IN, USA

ACKNOWLEDGEMENTS

We would like to thank Geoffrey A, Walford, and Michael Crutchlow from Merck & Co., Inc., Kenilworth, NJ, USA, for critical reading and feedback.

CONFLICT OF INTEREST

None declared.

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