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Pegbelfermin (BMS-986036)

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Pegbelfermin (BMS-986036): an investigational PEGylated fibroblast growth factor 21 analogue for the treatment of nonalcoholic steatohepatitis

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ABSTRACT

Introduction: Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide and is strongly associated with obesity and insulin resistance. NAFLD refers to a spectrum of disorders ranging from asymptomatic hepatic steatosis (nonalcoholic fatty liver, NAFL) to nonalcoholic steatohepatitis (NASH), which increases the risk of developing more severe forms of liver disease such as progressive fibrosis, cirrhosis, and liver cancer. Currently, there are no food and drug administration (FDA) approved drugs to treat NASH. Pegbelfermin (BMS-986036) is a PEGylated fibroblast growth factor 21 (FGF21) analogue that is under investigation for the treatment of NASH.

Areas covered: We reviewed the (pre)clinical pegbelfermin studies and compared these with other studies that assessed FGF21 and FGF21 analogues in the treatment of NASH.

Expert opinion: With no FDA approved treatments available for NASH, there is an urgent need for novel therapies. Pegbelfermin is a systemic treatment with pleiotropic effects on various tissues. Short-term adverse effects are limited, but more research is required to study potential long-term safety issues. In a phase 2a trial, pegbelfermin has shown promising improvements in several NASH related outcomes. However, clinical trials demonstrating long-term benefits on hard outcomes such as liver histology, cirrhosis development, or survival are required for further validation.

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Pegbelfermin; FGF21; NAFLD; NASH

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), a spectrum of disorders characterized by excessive liver fat accumulation in the absence of chronic alcohol abuse, is the leading cause of chronic liver disease worldwide [1]. Epidemiological studies indicate that NAFLD affects around 25% of the world population, with the highest prevalence reported in the Middle East (32%) and South America (30%) [2]. Of particular concern is that NAFLD is more commonly diagnosed in children as well and that global NAFLD prevalence is expected to increase even further in the near future [3].

In most cases, liver fat accumulation, also known as hepatic steatosis, is the result of an unhealthy lifestyle involving excessive energy intake and physical inactivity [4]. NAFLD is also regarded as the hepatic manifestation of the Metabolic Syndrome (MetS) as it is often observed alongside the morbidities that define this syndrome (*i.e.* central obesity, high blood pressure, high blood glucose, high serum triglycerides (TG), and low high-density lipoprotein (HDL) cholesterol) [2]. Other risk factors include age, male sex, and ethnicity [2]. Genetic predisposition also contributes to the development of NAFLD. Genome-wide association studies have shown that polymorphisms in patatin-like phospholipase domain-containing 3 (PNPLA3) and transmembrane 6 superfamily, member 2 (TM6SF2) are major genetic risk factors for NAFLD, as these polymorphisms increase hepatic TG content by about 25% in heterozygous and 200% in homozygous carriers compared to non-carriers [5,6]. However, the underlying mechanism by which these genes affect NAFLD risk is still unclear. Finally, it has become increasingly apparent that the gut microbiome may also play a role in NAFLD development by affecting intestinal permeability and short-chain fatty acid and ethanol production [7].

Ultimately, complex interactions between environmental and genetic factors result in accumulation of TG and other lipids in hepatocytes [8]. This increased intracellular lipid accumulation leads to lipotoxicity, mitochondrial dysfunction, and enhanced production of reactive oxygen species, which may result in significant amounts of cellular stress, cellular damage, and apoptosis [8]. Inflammatory, autophagic, endoplasmic reticulum stress, and hepatic stellate cell-driven fibrogenic pathways are subsequently activated to resolve cellular damage, resulting in the progression of steatosis toward nonalcoholic steatohepatitis (NASH) [8].

NAFL, which is histologically defined by the presence of macroscopic steatosis in more than 5% of hepatocytes, is considered to be a benign and reversible condition. However, around 25% of NAFL patients also exhibit NASH, in which steatosis is accompanied by histological signs of lobular inflammation and hepatocyte ballooning, with or without fibrosis [1,2]. The exact prevalence of NASH is likely higher, given that symptoms usually occur at an advanced disease stage and that NASH can only be reliably diagnosed by a liver biopsy, an invasive and risky procedure that is not performed on all NAFLD patients [2].

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Article Highlights

- NASH is a potentially life-threatening chronic liver disorder for which pharmacological treatments are currently not available.
- Pegbelfermin is a PEGylated FGF21 analogue with an increased half-life under investigation for the treatment of NASH
- In a phase 2a clinical trial, pegbelfermin has shown promising improvements in NASH-related outcomes, including a reduction in liver fat fraction and a decrease in markers of liver damage (ALT/ASAT) and fibrosis (PRO-C3).
- Short-term adverse health effects appear to be mild and mainly consist of injection site reactions.
- Further studies are needed to demonstrate improvements in liver histology or reduced progression to cirrhosis by pegbelfermin treatment.

This box summarizes key points contained in the article.

Box 1. Drug summary.

Drug name	Pegbelfermin (BMS-986036)
Phase	Phase 2b studies ongoing
Indication	Treatment of non-alcoholic steatohepatitis and liver fibrosis
Pharmacology description/ mechanism of action	FGF21 analogue that mediates its beneficial metabolic effects through FGF receptor 1c
Route of administration	Subcutaneous (abdomen)
Chemical structure	
Pivotal trials	Phase 2 studies on type 2 diabetes [45] (ClinicalTrials.gov Identifier: NCT02097277) and NASH [46] (ClinicalTrials.gov Identifier: NCT02413372) completed, phase 2b studies FALCON 1 (ClinicalTrials.gov Identifier: NCT03486899) and FALCON 2 (ClinicalTrials.gov Identifier: NCT03486912) currently ongoing

The progression of NASH can be classified according to several staging and grading systems based on the histological degree of steatosis, inflammation, and fibrosis [1]. Lipid accumulation and inflammation are believed to be essential drivers in the progression of NAFL to NASH. However, the occurrence of advanced liver fibrosis, or cirrhosis, which is present in 25% of NASH patients, is considered to be the most critical histological feature, as it is most strongly associated with long-term mortality [1,2]. Liver cirrhosis increases the risk of developing liver failure, hepatocellular carcinomas, and the need for liver transplantation. Therefore, treatments that can slow or stop the progression of NASH are highly needed.

2. Therapeutic options for NASH

2.1. Lifestyle changes

The first-line management of NASH involves lifestyle modifications directed toward weight loss. While a modest weight loss of 3-5% already causes a significant reduction in hepatic steatosis, more pronounced weight loss (7-10%) is needed to also reduce inflammation, cell damage, and fibrosis [2,9,10]. The most marked improvements in NASH resolution are seen in patients that achieved >10% weight loss [9]. However, it should be noted that >7% weight loss was only attained by 50% of patients, reflecting the well-known problem of reaching and maintaining sufficient weight loss [10]. Studies that examined the effects of exercise only in NASH indicate improvements in steatosis, while effects on inflammation and fibrosis remain unclear [11].

2.2. Bariatric surgery

Another effective way to lose weight and improve metabolic health is bariatric surgery. In one study, bariatric surgery led to NASH resolution in >85% of the patients one year after surgery, with improvements in fibrosis in 33% of patients [12]. These findings were supported by a recently performed meta-analysis showing that bariatric surgery led to a complete NASH resolution in 70% of the patients [13]. Although bariatric surgery appears to be a highly effective treatment for NASH, it is only performed on morbidly obese patients, and its use is limited by various contra-indications for undergoing surgery, such as heart failure, unstable coronary artery disease, end-stage lung disease, and portal hypertension [14].

2.3. Pharmacologic treatment

As lifestyle modifications and bariatric surgery are not feasible or effective in all NASH patients, there is an urgent need for novel (pharmacologic) treatments. Currently, several drugs targeting various biological pathways are in development. One of the front runners of these drugs, Ocaliva (OCA, INT-747, obeticholic acid, 6α-ethyl-chenodeoxycholic acid), has already entered phase 3 clinical trials for NASH treatment [15]. Ocaliva, a semi-synthetic analogue of the bile acid chenodeoxycholic acid (CDCA) and a potent FXR agonist developed by Intercept, has been shown to reduce hepatic steatosis and fibrosis in preclinical models [16,17]. In a proof of concept study in patients with NAFLD and type 2 diabetes mellitus (T2D), Ocaliva reduced liver damage, as determined by a reduction of serum γ-glutamyltransferase and alanine aminotransferase (ALT), and reduced fibrosis markers as determined by Enhanced Liver Fibrosis (ELF) scores, indicating its potential in NASH treatment. In addition, Ocaliva improved insulin sensitivity and weight loss [18]. The efficacy and safety of Ocaliva in the treatment of NASH in humans were evaluated in the FLINT trial (ClinicalTrials.gov ID: NCT01265498), a large multicenter phase 2b trial including 283 patients. This trial was ended at an early stage when the primary endpoint (improvement in the NAFLD activity score (NAS) score by at least 2 points with no worsening of liver fibrosis) was met in 45% of the Ocaliva treatment group and 21% of the placebo group [19]. Although Ocaliva was generally well-tolerated, adverse effects included an increase in pruritus (23% of the Ocaliva-treated patients *versus* 6% in the placebo group) as well as elevated levels of low-density lipoprotein (LDL) cholesterol [19]. Early 2019, an interim analysis of the phase 3 trial REGENERATE [20] (ClinicalTrials.gov ID: NCT02548351) reported that Ocaliva had achieved its primary endpoint demonstrating statistically significant improvement in liver fibrosis stage without worsening of NASH at 18 months at

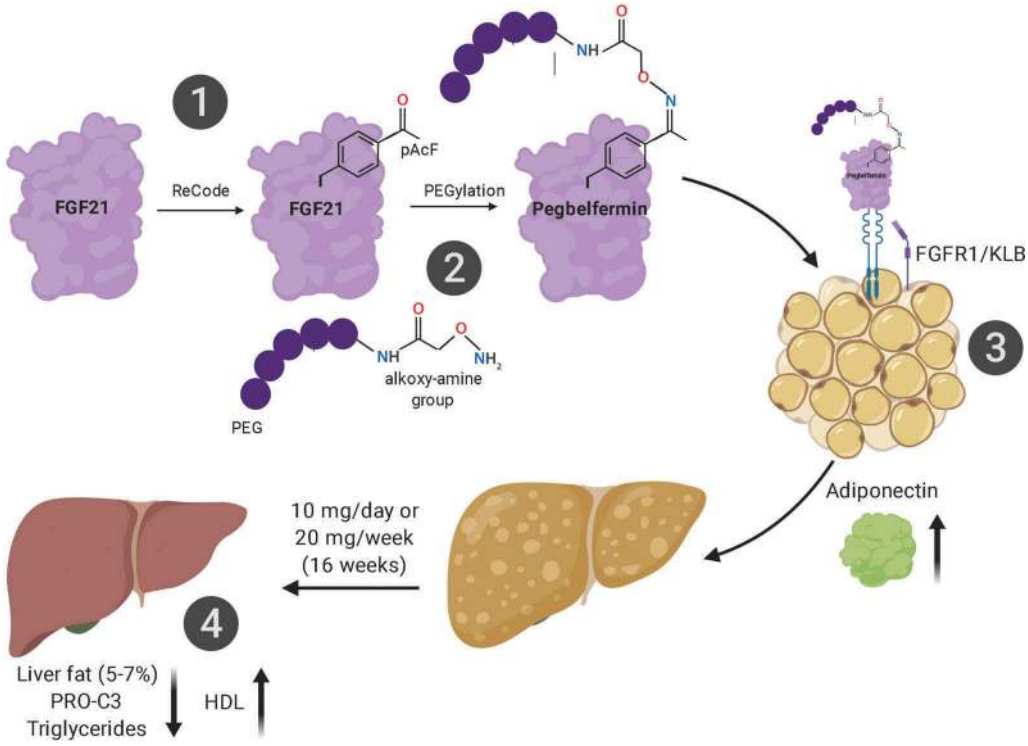

Figure 1. **Design and mechanism of action of pegbelfermin**. The stability of native FGF21 was increased by (1) adding a PEGylation site via insertion of the amino acid p-acetylphenylalanine (pAcF) using reconstituting chemically orthogonal-directed engineering (ReCode). This specific location of pAcF preserves receptor binding and minimizes loss of biological activity; (2) PEGylation was achieved by oxime bond formation between a PEG polymer containing an alkoxy-amine group and the ketone group on pAcF [44]. Preclinical studies suggest that PEGylated FGF21 will bind to the FGFR1/KLB complex in adipose tissue, leading to elevated secretion of the insulin-sensitizing hormone adiponectin, although additional metabolic pathways may be affected as well (3). Clinical studies have shown that pegbelfermin improves several NASH-related outcomes, including a decrease in liver fat content, plasma PRO-C3 levels, and plasma triglyceride levels, while plasma HDL-cholesterol levels increase (4) [45,46] .

a dose of 25 mg and 10 mg compared to placebo (23.1% vs 17.6% vs 11.9%) (presented at the International Liver Congress 2019). The second primary endpoint, which was NASH resolution without worsening of fibrosis, was not met. Consistent with the FLINT trial Ocaliva increased pruritus, and LDL cholesterol, indicating a potential safety issue.

2.3.1. Targeting metabolic hormone systems

Given that NASH is a multifactorial disease, a large number of biological pathways can potentially be modulated to reduce disease activity. Consequently, the effects of antioxidants (vitamin E), insulin sensitizers (pioglitazone), and immune modulators (cenicriviroc) in NASH treatment are being explored [4]. Direct targeting of endogenous hormone systems appears to be another feasible strategy to resolve NASH and reduce or reverse fibrosis progression. The gut-derived hormone glucagon-like peptide-1 (GLP-1) improves insulin secretion, suppresses appetite, lowers body weight, and delays gastric emptying [21]. Treatment with GLP-1 analogues ameliorates glycemic control and is also linked to lowering of serum ALT levels, suggesting that they have potential in NASH treatment [22]. In a small clinical trial (ClinicalTrials.gov ID: NCT01237119) of 52 patients, treatment with the GLP-1 analogue liraglutide led to NASH resolution, defined as the disappearance of hepatocyte ballooning, in 39% of the patients and significantly reduced the progression of fibrosis in 36% of the patients [22]. Treatment with liraglutide also affected components of MetS, including body weight loss and improved glycemic control [22].

Recently, several members of the fibroblast growth factor (FGF) family (*i.e*. FGF1, FGF19, and FGF21) have been identified as metabolic hormones with beneficial effects on NAFLD [23]. These FGFs are under transcriptional control of nuclear receptors (*i.e*. FXR regulates FGF19, whereas PPARγ and -α regulate FGF1 and −21, respectively). While native FGFs have several drawbacks for use in patients, including adverse effects and a short half-life, FGF analogues with improved pharmacokinetic and pharmacodynamic profiles are currently under development [24]. FGF19 is a hormone that is produced in the intestine and regulates bile acid production and energy metabolism. Pharmacologic administration of recombinant FGF19 shows remarkable preclinical efficacy in improving metabolic disorders such as T2D and NAFLD/NASH. However, because long-term FGF19 administration results in tumor formation in the liver, the development of a safe FGF19 variant was required, which culminated in the generation of NGM282, a non-mitogenic FGF19 variant that retains full metabolic activity [25]. Treatment of biopsy-confirmed NASH patients with NGM282 (ClinicalTrials.gov ID: NCT02443116), an analogue of the intestinal hormone FGF19, resulted in a clinically relevant decrease in hepatic fat content of ≥30%, determined by magnetic resonance imaging (MRI)-proton density fat fraction, in 86% the of patients, and was associated with a reduction in markers of liver damage (serum ALT and aspartate transaminase (AST)) and fibrosis (ELF score) [26]. NGM282 also improved histological features of NASH, including the

NAFLD activity score (NAS) by two or more points in 62% of the patients and fibrosis score by one stage or more in 52% of the patients receiving 3 mg once daily for 12 weeks compared to baseline (ClinicalTrials.gov ID: NCT02443116) [27].

2.4. FGF21 and FGF21-analogues

FGF21 is another member of the FGF-family and exerts its biological effects by binding to FGF receptors (FGFRs) in complex with the transmembrane protein β Klotho (KLB), resulting in the activation of various canonical signaling pathways such as mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase–protein kinase B/AKT (PI3K-PKB/AKT) [28]. FGF21 is primarily produced by the liver in response to metabolic stress, such as fasting or a ketogenic diet [29]. In mice, the induction of Fgf21 is associated with changes in lipolysis, ketogenesis, growth, torpor, and female reproduction, all responses related to the adaptive starvation response [29]. Whether FGF21 regulates similar functions in humans is still unclear. However, the identification of an FGF21 gene variant that is associated with increased sugar intake suggests that it has a role in the central regulation of carbohydrate consumption [30]. Association studies have provided additional evidence that FGF21 might play a role in metabolic regulation in humans as well. Increased plasma levels of FGF21 are associated with various obesity-related diseases, including T2D, coronary heart disease, and NAFLD/NASH [31,32]. Although it has been speculated that chronically elevated FGF21 levels reflect a state of FGF21 resistance, pharmacologic FGF21 administration has been shown to improve metabolic health in mice, non-human primates, and humans [33].

In diabetic mice, administration of recombinant FGF21 improved insulin sensitivity and dyslipidemia [34]. Chronic FGF21 treatment or FGF21 overexpression also protected against diet-induced obesity and was associated with enhanced insulin sensitivity in wild type mice [35]. Similar metabolic improvements were observed in diabetic primates [36]. The acute insulin-sensitizing effects of FGF21 are mechanistically linked to activation of the FGFR1/KLB complex in adipose tissue [37]. In contrast, the metabolic improvements of long-term FGF21 treatment, in particular weight loss, appear to be primarily caused by binding of FGF21 to the FGFR1/KLB complex in the brain [37]. The identification of these potent metabolic effects in pre-clinical studies has initiated the development of various FGF21-based drugs for the treatment of metabolic disease in humans.

FGF19 binds to FGFR4/KLB, which has been associated with HCC. In contrast, FGF21 does not bind to FGFR4/KLB and therefore, does not exert mitogenic effects. However, as native FGF21 has a short half-life and a tendency to aggregate, the focus has been on the development of analogues with improved pharmacokinetic properties [24,38]. LY2405319, the first FGF21-based drug tested in humans in a proof-of-concept trial, was shown to improve dyslipidemia and reduce body weight compared to baseline in patients with obesity and T2D (ClinicalTrials.gov ID: NCT01869959) [39]. PF-05231023, another FGF21 analogue consisting of two recombinant FGF21 molecules linked to the antigen-binding fragment of a scaffold antibody, significantly increased adiponectin levels, which may protect against steatosis and NASH [40], and decreased fasting plasma TG levels in two phase 1 studies in obese patients with T2D (ClinicalTrials.gov ID: NCT01396187 and NCT01673178) [41,42]. However, one of these studies found an increase in blood pressure and pulse frequency [42]. Currently, there are no ongoing registered clinical trials to further evaluate LY2405319 and PF-05231023 efficacy and safety.

The remainder of this review focuses on pegbelfermin (BMS-986036), a polyethylene glycol-modified (PEGylated) recombinant human FGF21 analogue with a prolonged half-life that is currently under investigation in clinical trials for the treatment of NASH. Figure 1 summarizes the design, mechanism of action and clinical effects of pegbelfermin.

3. Pegbelfermin (BMS-986036)

PEGylation of FGF21 increases the size and solubility and decreases proteolytic degradation of the molecule, resulting in a prolonged half-life and duration of action [43,44]. Pegbelfermin was initially developed by Ambrx Inc. (La Jolla, CA, USA) but has now been licensed to Bristol-Meyers Squibb. Two phase 2a trials with pegbelfermin in the treatment of obesity, T2D, and NASH have been completed [45,46]. Pegbelfermin is currently under evaluation in phase 2b trials in patients with NASH and liver fibrosis (FALCON 1 and FALCON 2 ClinicalTrials.gov Identifiers: NCT03486899 and NCT03486912, respectively).

3.1. Chemistry

The structure of FGF21 has been modeled based upon crystal structures of FGF19 and FGF23 [44]. By using a technique termed ReCode, a unique amino acid (p-acetyl phenylalanine, pAcF) was inserted at a specific site of FGF21 [47], to serve as conjugation site for PEGylation via oxime bond formation [44].

3.2. Pharmacodynamics

FGF21 binds efficiently to FGFR1c, FGFR2c, and FGFR3c in the presence of co-receptor KLB. While native FGF21 mediates its metabolic effects primarily via FGFR1c/KLB, in the presence of heparin, FGF21 can also bind to other FGFRs, although with considerably lower affinity [48,49]. The poor heparin-binding affinity of FGF21 results in an endocrine mode of action to signal in their target tissues [50]. In HEK293 cells that stably express KLB, a series of PEGylated FGF21 variants efficiently activated intracellular signaling, as determined by phosphorylation of extracellular signal-regulated kinase (ERK) [44]. The most potent PEGylated FGF21 variants resulted in a 5-fold increase of *in vitro* pERK activity compared to wild type FGF21 and induced glucose uptake in 3T3-L1 adipocytes [44]. Thus, the PEGylation of FGF21 potently enhances FGF21 signaling and functionality *in vitro*.

In vivo studies demonstrated that the PEGylation of FGF21 substantially prolongs the half-life in male rats receiving a single subcutaneous dose (0.25 mg/kg body weight) [44]. In the diabetic *db/db* mouse model, PEGylated FGF21 caused a dose-dependent lowering of fasting and ambient plasma glucose (−30 to 60%), without affecting body weight, at

doses of 0.75 and 2.5 mg/kg body weight administered once daily for 12 days. These results were similar to the effects of wild type FGF21 [44].

Preclinical studies evaluating the effect of pegbelfermin on NASH were conducted in the Stelic mouse model (STAM™). These mice develop features of NASH and liver fibrosis through a combination of chemical (streptozotocin) and dietary (high fat) interventions [51,52]. An important limitation of the STAM™ mouse model is that these mice do not develop insulin resistance and hyperinsulinemia but instead become insulin deficient [53]. Nine-week old STAM™ mice received a twice-weekly subcutaneous administration of 3 mg/kg body weight pegbelfermin for six weeks. After six weeks, pegbelfermin treatment resulted in improved survival and decreased liver-to-body weight ratio (−40%). Pegbelfermin also reduced hepatic TG and cholesterol (−50% and −51%, respectively), blood glucose levels (−37%), and plasma ALT levels compared to vehicle-treated mice [52]. Serum adiponectin levels increased, and the NAS decreased (−3.6 units) with reductions in all three components (steatosis, ballooning, and inflammation) [52]. Similarly, in a different study, STAM™ mice of 7 weeks of age received 3 mg/kg body weight pegbelfermin twice-weekly for two weeks by subcutaneous injection, which resulted in improved blood glucose levels (−30%), liver-to-body weight ratio (−20%), liver and plasma TG and lower mean NAS (−3.25 units) [54]. Pegbelfermin also decreased the serum fibrosis biomarker N-terminal type III collagen propeptide (PRO-C3) and reduced liver fibrosis quantified using a Sirius Red staining (−40%) [54].

In phase 1 studies, healthy obese subjects (body mass index (BMI) 30–40 kg/m^2) were randomized to receive pegbelfermin or placebo in single or multiple doses by subcutaneous administration. Subjects in the singe-dose group received one dose ranging from 0.3 to 60 mg pegbelfermin or placebo, while subjects in the multiple-dose group received doses ranging from 0.3 to 30 mg pegbelfermin or placebo once daily or 21 mg once weekly [55]. After two weeks, pegbelfermin treatment was dose-dependently associated with improvements in body weight, insulin sensitivity, serum lipids, and an increase in serum adiponectin, consistent with preclinical findings [55].

3.3. Pharmacokinetics and metabolism

The pharmacokinetics of pegbelfermin were first assessed in the phase 1 study mentioned above. Pegbelfermin was administered subcutaneously and showed linear pharmacokinetics with an average half-life elimination of 19–24 hours. Furthermore, pegbelfermin accumulated 2- to 3-fold in plasma when dosed daily, but accumulation in plasma was negligible when dosed weekly [55]. The pharmacokinetics of pegbelfermin during a phase 2 study, in which NASH patients received 10 mg pegbelfermin once a day or 20 mg pegbelfermin once a week subcutaneously, has not been published thus far [46]. The elimination route of pegbelfermin has not been described.

3.4. Clinical efficacy

Several FGF21 analogues have been investigated as a treatment for obesity, T2D, and NAFLD in phase 1 and phase 2 clinical trials [39,41,42,45,46,55,56]. Table 1 summarizes the most important outcomes of the phase 2 trials of FGF21 analogues on body weight and metabolic parameters in plasma and liver, compared to placebo.

The efficacy of pegbelfermin has been studied in obese patients with T2D [45] and patients with biopsy-confirmed NASH (fibrosis stage 1–3) [46]. While improvements in histology, the gold standard for demonstrating short-term improvements in NAFLD/NASH, have not been demonstrated, Sanyal *et al*. showed several benefits on other NAFLD biomarkers [46]. Primary outcome measures were a change in hepatic fat fraction and safety parameters after 16 weeks of treatment compared to placebo. Both daily (10 mg) and weekly (20 mg) subcutaneous injections of pegbelfermin reduced hepatic fat content as measured by MRI [46]. The reduction in liver fat was robust after 16 weeks of pegbelfermin treatment (5-7% in

Table 1. The effects of current FGF21 analogs on several metabolic parameters in plasma and liver. *at higher doses, body weight was significantly lower compared to baseline but not to placebo. **daily dosage of Pegbelfermin seemed to reduce LDL-C while the weekly dosage did not. ***while more patients on Pegbelfermin treatment had a greater than 15% reduction in MRE measured liver stiffness, statistical significance could not be assessed due to a small sample size.

	LY2405319 [39]	*PF-05231023 [41,42]*	*Pegbelfermin (BMS-986,036) [45,46]*
Dosage	*3,10,20 mg/day*	*5,25,100,140 mg 2x/week [41]* *25,50,100,150 mg/week [42]*	*1,5,10,20 mg/day or 20mg/week [45]* *10 mg/day or 20mg/week [46]*
Duration	*4 weeks*	*4 weeks [41,42]*	*12 weeks [45]* *16 weeks [46]*
Group size	10-15	8-12 [41] 21–22 [42]	24 [45] 24–26 [46]
Body weight	↔*	↓ [41], ↔ [42]	↔ [45]
Plasma parameters			
Glucose/insulin resistance	↔	↔ [41,42]	↔/↓ [45]
Insulin	↔	↔ [41,42]	↔ [45]
Adiponectin	↑	↑ [41,42]	↑ [45,46]
Triglycerides	↓	↓ [41,42]	↓ [45,46]
LDL-C	↓	↓ [41,42]	↔** [45,46]
HDL-C	↑	↑ [41,42]	↑ [45,46]
PRO-C3			↓ [45,46]
ALT/ASAT			↓[48]
Liver parameters			
Fat fraction (MRI)			↓ [46]
Liver stiffness (MRE)			↔/↓*** [46]

treated groups compared to 1% in the placebo group). Both, Sanyal *et al*. and Charles *et al*. showed decreases in serum PRO-C3 at dosages of either 10–20 mg daily or 20 mg weekly [45,46]. Serum PRO-C3 is a biomarker for collagen formation and has been shown to highly correlate with the presence of advanced fibrosis in NAFLD, especially when combined with an algorithm including several other risk factors [57]. Liver stiffness by magnetic resonance elastography (MRE) was only measured in a small subset of patients (placebo, n = 14; 10 mg QD, n = 11; and 20 mg QW, n = 12) in the study by Sanyal *et al*., therefore, no firm conclusion can be drawn [46]. While pegbelfermin treatment showed no apparent improvement in liver stiffness compared to placebo, more patients on pegbelfermin showed a higher relative reduction (of at least 15%).

Currently, only one study has been specifically designed to address the clinical efficacy of pegbelfermin in the treatment of NASH [46]. While this study showed promising results on steatosis and plasma PRO-C3 levels, it also had clear limitations in terms of sample size, outcomes, and duration. More, longer duration, studies are therefore required to assess the effects on liver histology and long-term NASH related complications. Trials investigating the histologic effects of weekly dosing of pegbelfermin are ongoing and expected to be completed in 2021 (clinicaltrials.gov identifiers: NCT03486899 and NCT03486912).

3.5. Safety and tolerability

Given that FGF21 is a hormone with pleiotropic effects on various tissues, caution is warranted when assessing its potential in the treatment of NASH and related metabolic conditions, as these likely require long-term administration. Compared to therapies based on other FGFs, such as FGF1 and FGF19, FGF21 has the advantage of being non-mitogenic [23,58]. Mice with transgenic overexpression of FGF21 even display growth inhibition due to reduced growth hormone signaling [59]. FGF21 also exerts cerebral effects in mice resulting in changes in behavior and glucocorticoid levels [60]. In mice, the effects of FGF21 on bone loss are mixed. In one study, FGF21 was found to inhibit osteoblastogenesis and stimulate adipogenesis from bone marrow, thereby increasing bone loss [61]. However, in another murine study, FGF21 did not affect bone homeostasis under similar experimental conditions [62]. In humans, the FGF21 analogue PF-05231023 also lowered several biomarkers of bone formation such as osteocalcin, a surrogate marker for osteoblast activity [41]. In both phase 2 studies with pegbelfermin, no data on biomarkers of bone formation were presented, but bone mineral density, measured by dual-energy x-ray absorptiometry, was unchanged at the end of treatment and six months after the end of treatment [45,46].

Short-term adverse effects of pegbelfermin treatment were generally mild and limited [45,46,55]. Most reported adverse effects were injection site reactions such as bruising or erythema, but these were not different from placebo injections [45,46]. Other more frequently reported adverse events included gastrointestinal symptoms such as nausea, dyspepsia, and diarrhea. These adverse effects were slightly higher in some pegbelfermin treated groups compared to placebo but did not show a dose-dependency [45,46]. Another potential safety concern of pegbelfermin is the PEGylation itself, which can lead to tissue vacuole formation in the renal tubular epithelium, and is of particular concern for the treatment of T2D patients with renal insufficiency [63].

It should also be noted that in the study by Sanyal *et al*., 63% of the 20 mg weekly group and 92% of the 10 mg daily group developed anti-pegbelfermin and anti-FGF21 antibodies [46]. Although antibody titers were generally low and not associated with (immune-related) adverse effects or pharmacokinetic/dynamic changes, it is crucial for future studies to follow-up on possible long-term consequences and dynamics of these immunological observations.

3.6. Regulatory affairs

Several drugs are currently under investigation and entering phase 2 or 3 trials for the treatment of NASH [64]. For novel NASH drugs to be accepted for full approval, the FDA requires improvements in hard clinical outcomes, including reduced liver-related events such as hepatic decompensation or liver transplantation, as well as overall mortality [65]. Showing benefits on those hard clinical endpoints requires large and long-term randomized control trials. However, with no FDA approved drugs and the growing magnitude of the clinical burden of NASH worldwide, histological improvements that reasonably likely predict clinical benefits, will support accelerated approval. The ongoing phase 2b trials on pegbelfermin combined with current data could provide the basis for moving into phase 3 trials and consequently, potential FDA approval.

4. Conclusion

Currently, there are no FDA approved drugs for the treatment of NASH. In fact, according to a recently performed meta-analysis aimed to assess the relative benefits and harms of drugs for NAFLD/NASH, there is no evidence that any current pharmacological treatment can reduce mortality, cirrhosis, decompensated cirrhosis, or the need for liver transplantation [66]. Results from preclinical and clinical studies on pegbelfermin have highlighted it as a candidate to resolve NASH and reduce fibrosis progression. The administration of 10–20 mg per day or 20 mg once a week is sufficient to improve several NASH related parameters both in mice and humans. The overall metabolic improvements are in line with the effects of native FGF21 and other analogues tested in preclinical and clinical studies. The main benefit of pegbelfermin over other FGF21 analogues is its PEGylation which increases the half-life time, leading to more sustained effects. Pegbelfermin was generally well-tolerated in the first human trials with injection site reactions as the main side effect [55]. Results from phase 3 clinical trials are required to assess the long-term safety and effects on hepatic steatosis, NASH disease activity (inflammation and ballooning), and fibrosis.

5. Expert opinion

Pegbelfermin is a systemic treatment with pleiotropic effects on various tissues. Although pegbelfermin has shown promising improvements in several NASH-related outcomes in short-term phase 2a trials, more research is required. Currently, there are no

generally accepted surrogate endpoints for NASH [67]. For demonstrating treatment benefits in NASH, the gold standard is performing liver biopsies and showing either short-term improvements in histology or long-term reduced progression to cirrhosis and its complications. Although conditional FDA approval can be achieved by showing improvements in histology, hard long-term outcomes on liver failure, liver transplantation, and survival are even more relevant. Thus far, none of the pegbelfermin studies have shown either histological improvements or long-term endpoints.

Pegbelfermin treatment resulted in a reduction of liver fat of 5-7% after 16 weeks, which was measured using MRI. However, the clinical relevance of this observation remains unclear as there is no direct relation between the degree of hepatic steatosis and clinically relevant fibrosis or NASH [68]. Short-term adverse effects of pegbelfermin are mild, but long-term safety concerns regarding bone mineral density and immunogenicity, including anti-pegbelfermin or anti-FGF21 antibodies, and the potential adverse effects of PEGylation itself still need to be assessed.

Taken together, pegbelfermin shows potential in the management of NASH, and adverse health effects appear to be mild. In the coming years, new clinical trials evaluating pegbelfermin will have been completed and will provide more pivotal data regarding histology, effectiveness and safety, which may lead to conditional FDA approval to resolve NASH and fibrosis progression. In the meantime, it should become clear whether other FGF21 mimetics, such as the anti-FGFR1/βKlotho antibody bFKB1 [37] or the FGF21/FGF1 chimera $FGF1^{\Delta HBS}$-$FGF21^{C\text{-}tail}$ [69] can also find their way from bench to bedside.

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Declaration of interest

JW Jonker holds a US patent (20190358296), 'Methods for treating metabolic disorders using FGF'. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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