Preclinical efficacy and clinical translatability of long-acting FGF-21 analogue in the GAN diet-induced obese and biopsy-confirmed mouse model of NASH

Authors
Kristoffer Voldum-Clausen, Jacob Nøhr-Meldgaard, Susanne Pors, Henrik H. Hansen, Michael Feigh

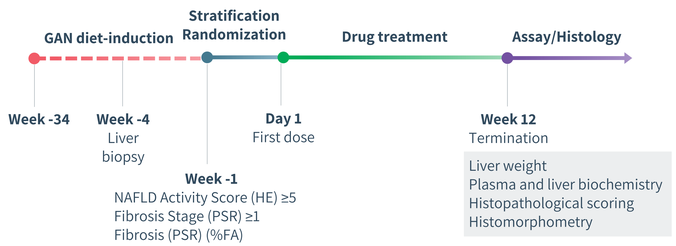
Gubra, Hørsholm, Denmark

Corresponding author
Michael Feigh - mfe@gubra.dk

BACKGROUND & AIM

Fibroblast growth factor 21 (FGF-21) plays a key role in hepatic lipid metabolism and holds great promise as therapeutic target for non-alcoholic steatohepatitis (NASH). The long-acting FGF-21 analogues efruxifermin and pegozafermin has in two recent phase 2 clinical trials (HARMONY phase 2b trial, press release Sept. 13, 2022), ENLIVEN phase 2b trial, press release March 22, 2023) demonstrated promising efficacy for both NASH resolution and improvement in fibrosis stage as compared to placebo controls. The present study aimed to *(i)* evaluate the metabolic, biochemical and histopathological effects of the long-acting FGF21 analogue PF-05231023 in the Gubra-Amylin NASH (GAN) diet-induced obese (DIO) mouse model of fibrosing NASH; and *(ii)* compare to primary outcomes of recent clinical phase-2 trial data.

1 Study outline



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dose
1	LEAN-CHOW	Male	6	**Vehicle**	SC	BIW	-
2	DIO-NASH	Male	14	**Vehicle**	SC	BIW	-
3	DIO-NASH	Male	14	**FGF21**	SC	BIW	10mg/kg

2 Metabolic and biochemical parameters

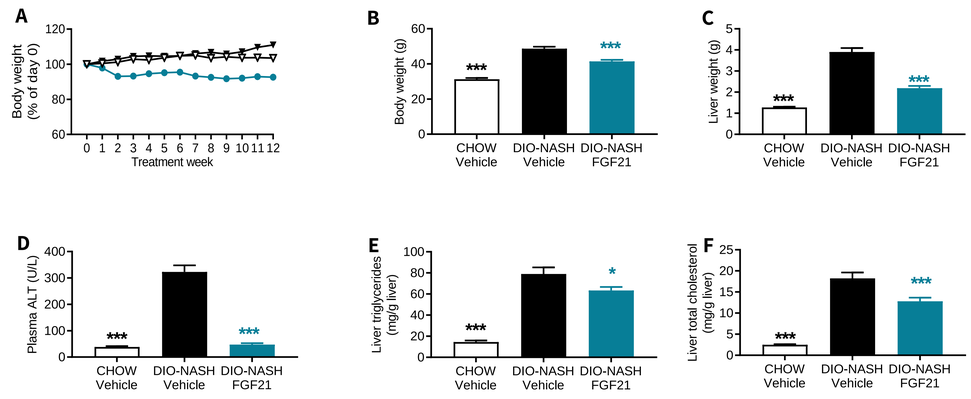


Figure 1. FGF21 (PF-05231023) improves hepatomegaly and biochemical parameters in GAN DIO-NASH mice. **(A)** Body weight change relative to baseline (day 0). **(B)** Terminal body weight (g). **(C)** Terminal liver weight. **(D)** Terminal plasma alanine aminotransferase (ALT). **(E)** Terminal liver triglycerides. **(F)** Terminal liver total cholesterol. *p<0.05, **p<0.01, ***p<0.001 compared to corresponding DIO-NASH vehicle control (Dunnett's test one-factor linear model).

3 NAFLD Activity Score and Fibrosis stage

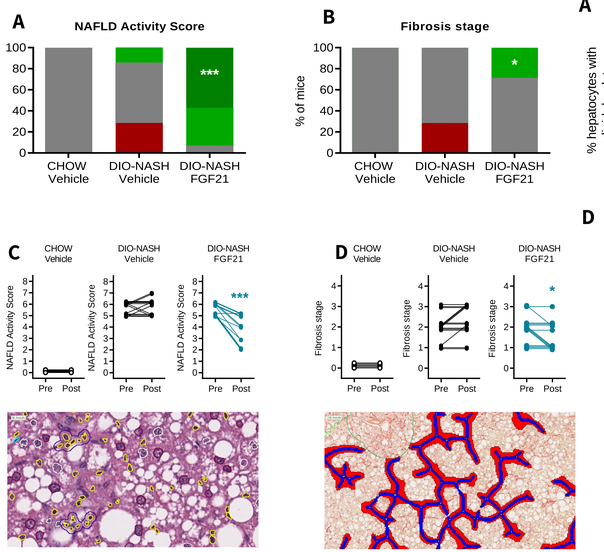


Figure 2. FGF21 (PF-05231023) improves liver histopathological scores in GAN DIO-NASH mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis stage. **(C)** Comparison of individual pre-post NAS and individual pre-post Fibrosis stage. *p<0.05, ***p<0.001 to corresponding DIO-NASH vehicle group (One-sided Fisher's exact test with Bonferroni correction). Bottom panels: Representative HE and PSR photomicrographs used for GHOST evaluation.

4 Quantitative histological markers of steatosis, inflammation and fibrosis

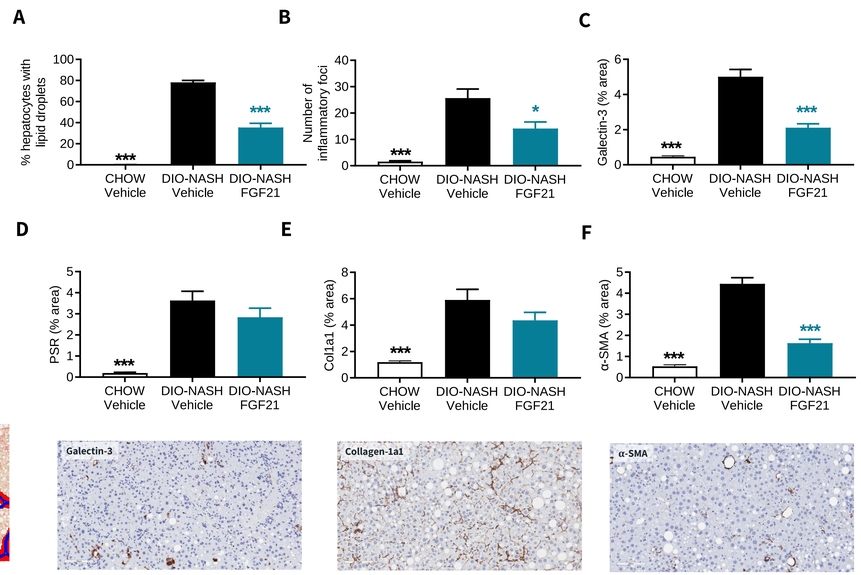
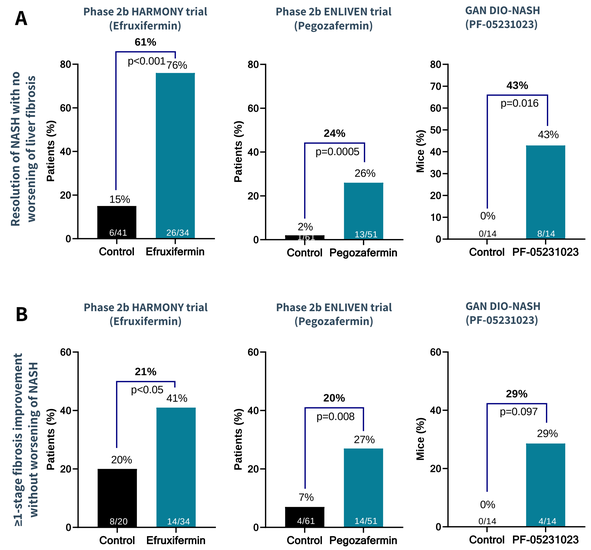


Figure 3. FGF21 (PF-05231023) improves histological markers of steatosis, inflammation and fibrogenesis in GAN DIO-NASH mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). **(A)** % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of galectin-3. **(D)** % area of PSR. **(E)** % area of collagen-1a1. **(F)** % area of alpha-smooth muscle actin (α-SMA) as marker for stellate cell activation (fibrogenesis). Mean ± SEM. *p<0.05, ***p<0.001 to corresponding DIO-NASH vehicle group (Dunnett's test one-factor linear model). Bottom panels: Representative galectin-3, collagen 1a1 and α-SMA photomicrographs for FGF21 treatment group (scale bar, 100 µm).

5 Clinical translatability



Figur 4. Comparison of FGF21 analogue treatment outcomes in GAN DIO-NASH mice vs. clinical trials for NASH. Efruxifermin (AKR-001, Fc-IgG1 conjugated FGF-21 analogue); Pegozafermin (BIO89-100, glycoPEGylated FGF21 analogue); PF-05231023 (CVX-343, antibody scaffold-conjugated FGF21 analogue). **(A)** Resolution of NASH (inflammation score ≤1; hepatocyte ballooning=0, with at least a 2-point reduction in NAS) with no worsening of liver fibrosis for Efruxifermin (HARMONY phase 2b trial, 50 mg, Q1W, 24 weeks, press release Sept. 13, 2022), Pegozafermin (ENLIVEN phase 2b trial, 44 mg Q2W, 24, weeks, press release March 22, 2023), and PF-05231023 (GAN DIO-NASH mice, 10 mg/kg , SC, QD, 12 weeks). **(B)** ≥1-stage fibrosis improvement without worsening of NASH for Efruxifermin, Pegozafermin and PF-05231023. *p<0.05, ***p<0.001 compared to vehicle-dosed controls (Fisher's exact test, Dunnett's test one-factor linear model).

CONCLUSION

- FGF21 (PF-05231023) reduces hepatomegaly, plasma ALT, liver triglycerides and liver total cholesterol.
- FGF21 (PF-05231023) promotes ≥2-point significant improvement in NAFLD Activity Score.
- FGF21 (PF-05231023) promotes 1-point significant improvement in Fibrosis Stage.
- FGF21 (PF-05231023) reduces quantitative histological markers of steatosis, inflammation and fibrogenesis.
- These findings agree with clinical phase 2b trial findings, further highlighting clinical translatability of the GAN DIO-NASH mouse model.

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