Metabolic, biochemical, histological, and transcriptomic effects of a long-acting FGF-21 analogue in the GAN diet-induced obese and biopsy-confirmed mouse model of NASH

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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 analogue efruxifermin has in a recent phase 2 clinical trial demonstrated promising efficacy for both NASH resolution and improvement in fibrosis stage as compared to placebo controls. The present study aimed to evaluate the therapeutic efficacy of the long-acting FGF21 analogue PF-05231023 in the GAN (Gubra-Amylin NASH) diet-induced obese (DIO) mouse model of NASH with hepatic fibrosis.

Study outline

Group #	Animal	Gender	Strain	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing volume	Dosing concentration
1	LEAN-CHOW	Male	C57BL/6JRj	6	Vehicle	SC	BIW	5 ml/kg	-
2	DIO-NASH	Male	C57BL/6JRj	14	Vehicle	SC	BIW	5 ml/kg	-
3	DIO-NASH	Male	C57BL/6JRj	14	FGF21	SC	BIW	5 ml/kg	10 mg/kg

Improvement in metabolic and biochemical parameters

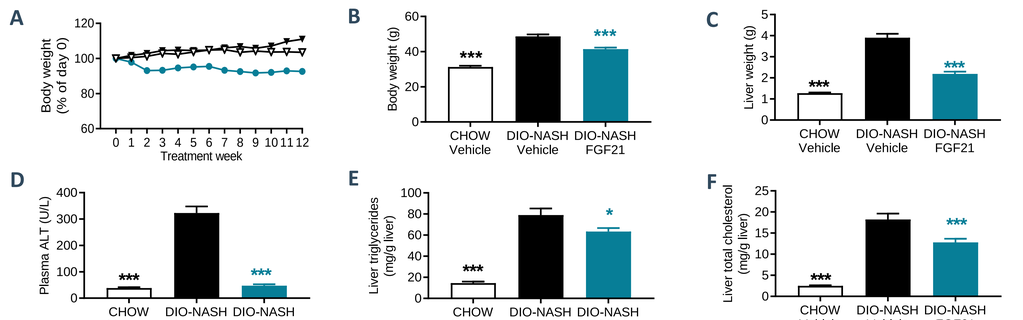

Figure 1. FGF21 (PF-05231023) improves hepatomegaly and biochemical parameters in GAN DIO-NASH mice. **(A)** Body weight change relative to baseline (day 0). **(B)** Terminal body weight (g). **(C)** Terminal liver weight. **(D)** Terminal plasma alanine aminotransferase (ALT). **(E)** Terminal liver 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).

Improvement in NAFLD Activity Score and Fibrosis Stage

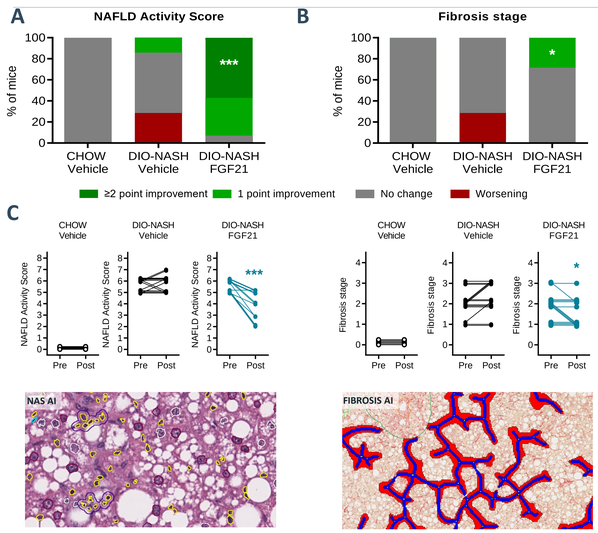

Figure 2. FGF21 (PF-05231023) improves liver histopathological scores in GAN DIO-NASH mice.
Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis stage. **(C)** Comparison 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.

Improvement in quantitative histology of steatosis, inflammation and fibrogenesis

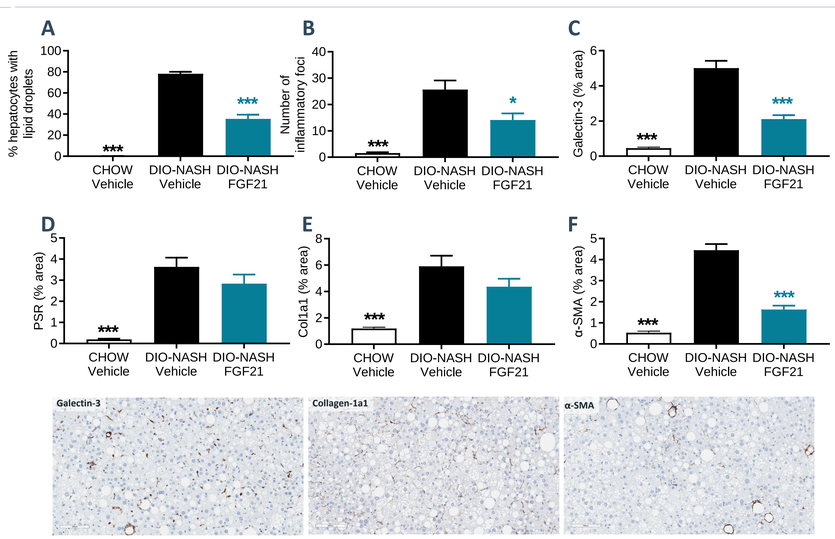

Figure 3. FGF21 (PF-05231023) improves quantitative liver histological markers in GAN DIO-NASH mice.
Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). **(A)** % hepatocytes 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. 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).

Hepatic transcriptomic profile for fibrosis and inflammation

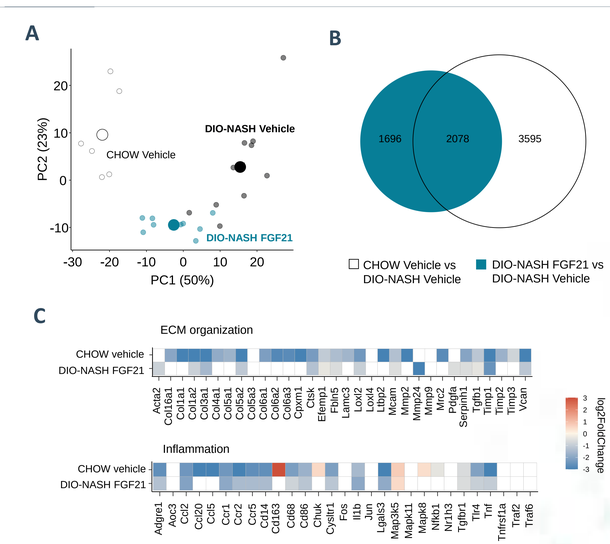

Figure 4. FGF21 (PF-05231023) suppressed inflammation-associated genes in GAN DIO-NASH mice.
(A) Principal component analysis (PCA) of samples based on top 500 most variable gene expression levels. **(B)** Venn diagram depicting shared and separate differentially expressed genes in treatment groups. **(C)** Regulation of hepatic extracellular matrix (ECM) candidate genes (log2-fold change compared to DIO-NASH vehicle mice). Blue colour gradients indicate significantly (p<0.05) down-regulated gene expression. White boxes indicate genes not significantly regulated (p>0.05) compared to DIO-NASH vehicle mice.

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 stellate cell activation.
- FGF21 (PF-05231023) suppresses inflammation-associated gene expression.
- These findings agree with clinical findings, further highlighting clinical translatability of the GAN DIO-NASH mouse model.