Hepatoprotective effects of efruxifermin in the GAN diet-induced obese and biopsy-confirmed mouse model of MASH

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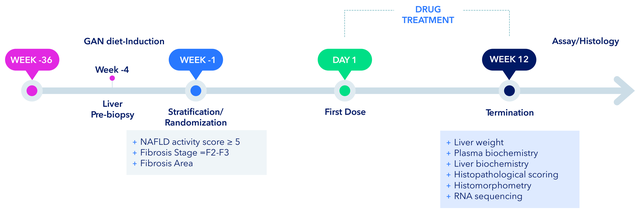
Background & Aim

Efruxifermin (EFX, long-acting FGF21 analogue) is currently in phase-3 clinical development for the treatment of metabolic dysfunction-associated steatohepatitis (MASH). The present study aimed to investigate the therapeutic benefits of EFX on metabolic, biochemical, histopathological and transcriptome profile in the Gubra Amylin NASH (GAN) diet-induced obese (DIO) and biopsy-confirmed mouse model of MASH with liver fibrosis.

1 Study outline



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing concentration
1	LEAN- CHOW	Male	10	**Vehicle**	SC	QW	-
2	DIO-MASH	Male	17	**Vehicle**	SC	QW	-
3	DIO-MASH	Male	17	**EFX**	SC	QW	1mg/kg

Figure 1. Study outline. SC; subcutaneous, QW; Once Weekly; GAN; Gubra Amylin NASH.

2 Metabolic and biochemical parameters

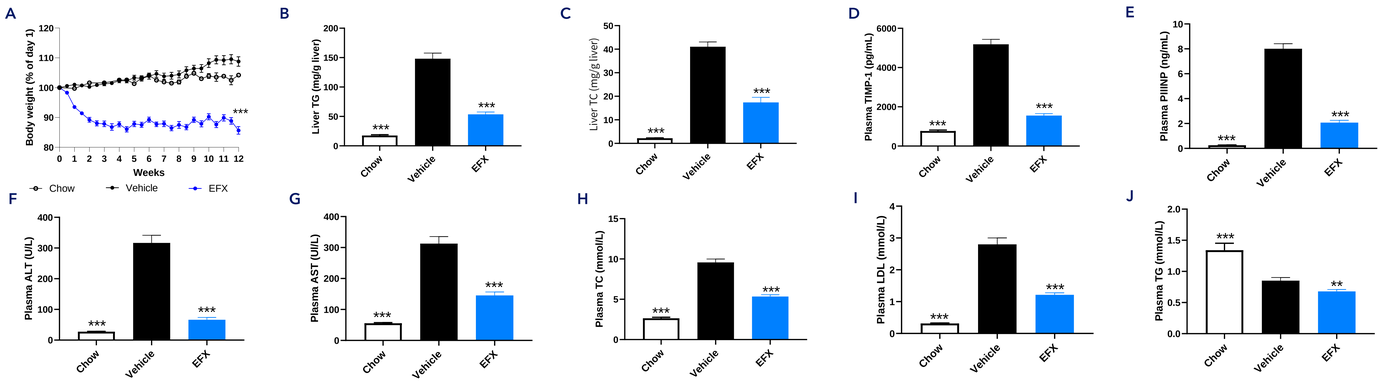


Figure 2. Metabolic and biochemical effects of EFX. (A) Relative body weight during study period. **(B)** Liver triglycerides (TG). **(C)** Liver total cholesterol (TC). **(D)** Plasma TIMP-1. **(E)** Plasma PIIINP **(F)** Plasma alanine aminotransferase (ALT). **(G)** Plasma alanine transaminase (AST). **(H)** Plasma total cholesterol (TC). **(I)** Plasma LDL cholesterol. **(J)** Plasma triglycerides (TG).** p<0.01, ***p<0.001 compared to vehicle control (Dunnett's test one-factor linear model).

3 NAFLD Activity Score and Fibrosis Stage

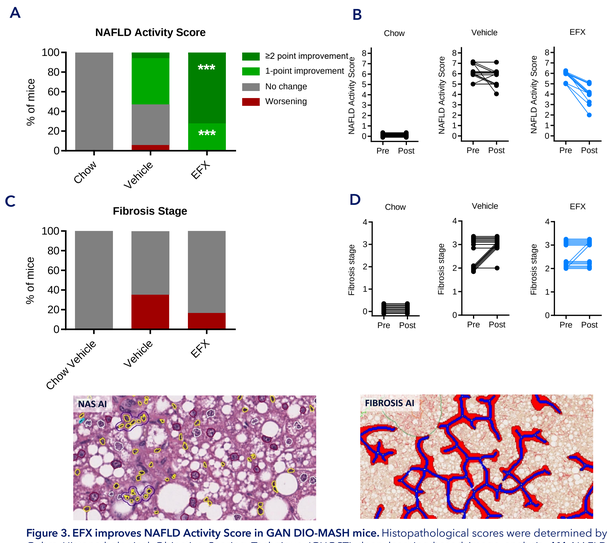


Figure 3. EFX improves NAFLD Activity Score in GAN DIO-MASH mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Individual pre-post NAS. **(C)** Fibrosis stage. **(D)** Individual pre-post fibrosis stage. ***p<0.001 compared to vehicle control (one-sided Fisher's exact test).

4 Histological markers of steatosis, inflammation, fibrosis and fibrogenesis

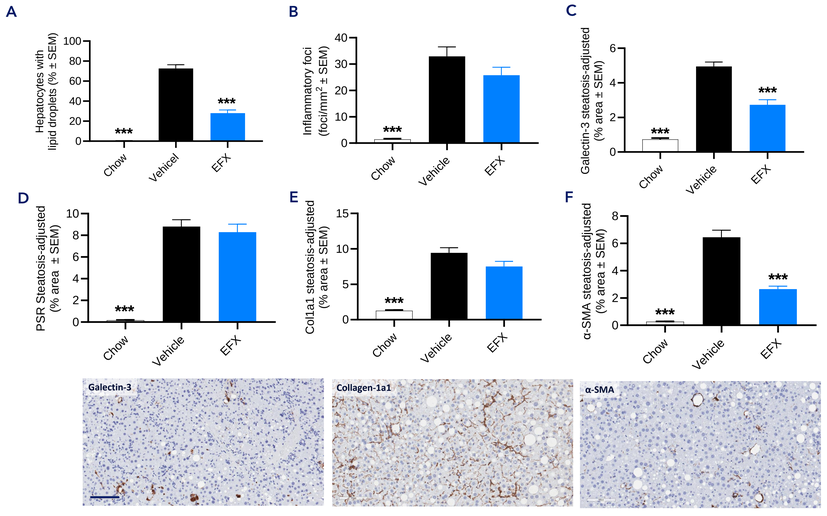


Figure 4. EFX improves quantitative histological endpoints. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). **(A)** % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of galectin-3 (steatosis-adjusted). **(D)** % area of PSR (steatosis-adjusted). **(E)** % area of collagen-1a1 (Col1a1, steatosis-adjusted) **(F)** % area of alpha-smooth muscle actin (α-SMA, marker of stellate cell activation; steatosis-adjusted). ***p<0.001 compared to vehicle control (Dunnett's test one-factor linear model). Bottom panels: Representative photomicrographs of galectin-3, Col1a1 and α-SMA (scale bar, 100 µm).

5 Liver transcriptome analysis

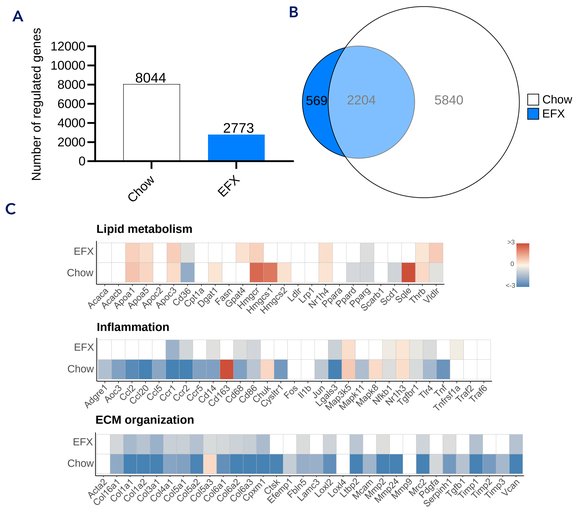


Figure 5. Hepatic transcriptome signatures following EFX therapy. (A) Total number of differentially expressed genes. **(B)** Venn diagram on overlapping gene expression signatures. **(C)** Regulation of gene expression markers of hepatic lipid metabolism, inflammation and extracellular matrix (ECM). Log2 fold change is indicated for significantly regulated genes (p_{adj} < 0.05 after correcting for multiple testing). Red and blue colours indicate up- and down-regulation, respectively, compared to Vehicle.

Conclusion

- EFX improves body weight, hepatomegaly, plasma transaminases and lipid levels.
- EFX improves NAFLD Activity Score, but not Fibrosis Stage.
- Benefits on NAS are supported by quantitative histological markers for steatosis and inflammation.
- EFX reduces quantitative histological markers for fibrogenesis and plasma markers of fibrosis.
- EFX regulates hepatic transcriptomic profile, improving gene expression markers of lipid metabolism and fibrogenesis.
- Longer treatment intervention might be needed for demonstrating histological anti-fibrotic effects of EFX.
- The hepatoprotective effects observed for EFX further supports clinical translatability of the biopsy-confirmed GAN DIO-MASH mouse model.