Characterization of lanifibranor treatment in the non-obese CDAA-HFD mouse model of advanced NASH with progressive fibrosis

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BACKGROUND & AIM

The pan peroxisome proliferator-activated receptor (PPAR-α/δ/ɣ)) agonist has recently been reported to improve liver histological outcomes in patients with non-alcoholic steatohepatitis (NASH) and fibrosis (NATIVE study; Francque et al, NEJM, 2021). Lanifibranor is currently in phase-3 clinical trial (NATiV3) for the treatment of NASH.

We have recently characterized lanifibranor treatment in the translational GAN diet-induced obese (DIO) mouse model of fibrosing NASH (Møllerhøj et al. Clin Transl Sci, 2022). The present study aimed to evaluate lanifibranor treatment in the non-obese choline-deficient L-amino-acid defined high-fat diet (CDAA-HFD) mouse model of advanced NASH with progressive fibrosis.

METHODS

C57BL/6JRj mice were fed chow or choline-deficient high-fat diet (CDAA-HFD, 45 kcal% fat, 0.1% methionine, 1% cholesterol, 28 kcal% fructose) for 6 weeks before treatment start (i.e. after induction of fibrosis). Prior to treatment, animals were randomized into treatment groups based on body weight. A baseline group (n=12) was terminated at study start. CDAA-HFD fed mice (n=12 per group) received treatment (PO, QD) with vehicle or lanifibranor (30 mg/kg) for 8 weeks. Chow-fed mice (n=8) served as normal controls.
Terminal endpoints included plasma and liver biochemistry, NAFLD Activity Score (NAS), fibrosis stage quantitative liver histology and transcriptome signatures.

1 Study outline

Group no.	Group	Name	Number of animals
1	Chow	Chow	8
2	Baseline CDAA-HFD	Baseline	12
3	Vehicle CDAA-HFD	Vehicle	12
4	Lanifibranor CDAA-HFD	Lanifibranor	12

2 Metabolic and biochemical parameters

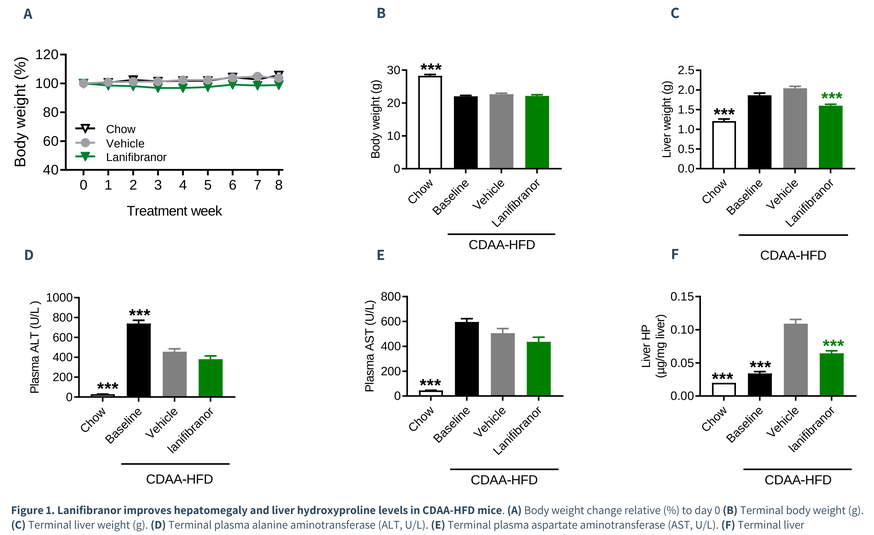

Figure 1. Lanifibranor improves hepatomegaly and liver hydroxyproline levels in CDAA-HFD mice. **(A)** Body weight change relative (%) to day 0 **(B)** Terminal body weight (g). **(C)** Terminal liver weight (g). **(D)** Terminal plasma alanine aminotransferase (ALT, U/L). **(E)** Terminal plasma aspartate aminotransferase (AST, U/L). **(F)** Terminal liver hydroxyproline (HP, µg/mg). ***p<0.001 compared to corresponding CDAA-HFD vehicle group (Dunnett's test one-factor linear model).

3 NAFLD Activity Score and Fibrosis Stage

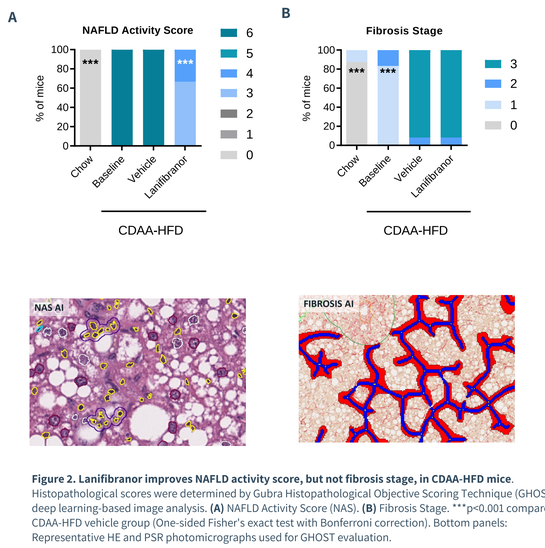

Figure 2. Lanifibranor improves NAFLD activity score, but not fibrosis stage, in CDAA-HFD mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis Stage. ***p<0.001 compared to CDAA-HFD 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 fibrogenesis

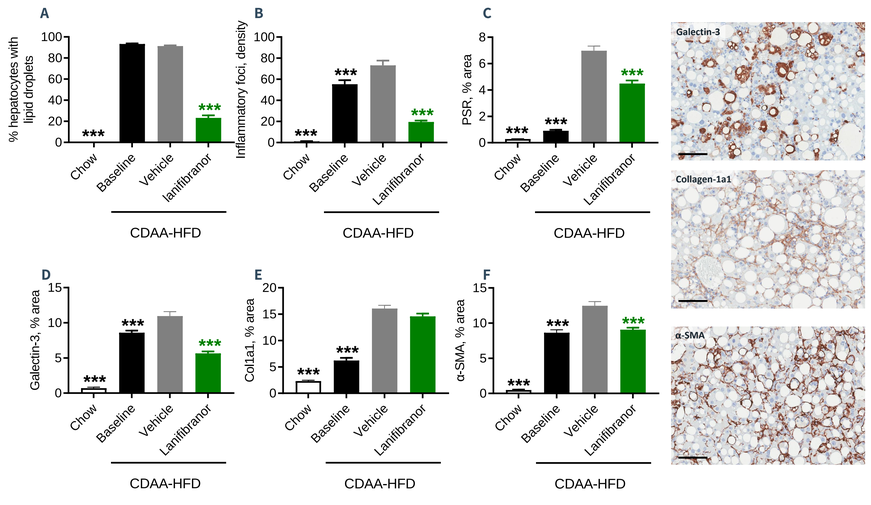

Figure 3. Lanifibranor improves quantitative histological markers of steatosis, inflammation and fibrogenesis in CDAA-HFD mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables and conventional IHC image analysis **(A)** % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of PSR. **(D)** % area of galectin-3. **(E)** % area of collagen-1a1. **(F)** % area of alpha-smooth muscle actin (α-SMA). Mean ± SEM. ***p<0.001 compared to CDAA-HFD vehicle group (Dunnett's test one-factor linear model). Right panels: Representative galectin-3, collagen 1a1 and α-SMA photomicrographs (scale bar, 100 µm).

5 Liver transcriptome profile

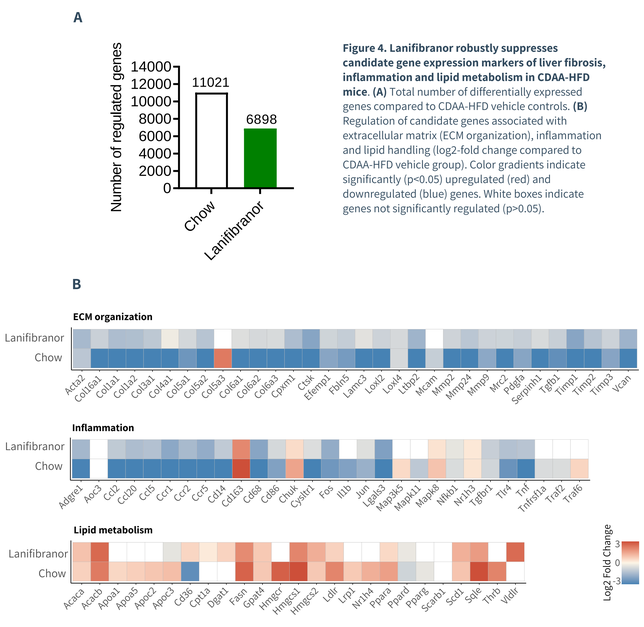

Figure 4. Lanifibranor robustly suppresses candidate gene expression markers of liver fibrosis, inflammation and lipid metabolism in CDAA-HFD mice. **(A)** Total number of differentially expressed genes compared to CDAA-HFD vehicle controls. **(B)** Regulation of candidate genes associated with extracellular matrix (ECM organization), inflammation and lipid handling (log2-fold change compared to CDAA-HFD vehicle group). Color gradients indicate significantly (p<0.05) upregulated (red) and downregulated (blue) genes. White boxes indicate genes not significantly regulated (p>0.05).

CONCLUSION

Lanifibranor treatment in CDAA-HFD mice:

+ Reduces hepatomegaly and liver hydroxyproline levels
+ Improves NAFLD Activity Score
+ Shows no effect on fibrosis stage
+ Reduces quantitative histological markers of steatosis, inflammation and fibrosis
+ Suppresses hepatic genes linked to inflammation and fibrosis

Effects of lanifibranor treatment in the non-obese CDAA-HFD mouse model of NASH with progressive fibrosis are in partial agreement with clinical trial outcomes in NASH patients.