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

Authors
Jacob Nøhr-Meldgaard, Ditte D. Thorbeck, Maja W. Andersen, Denise Oro, Henrik H. Hansen, Michael Feigh.

Gubra, Hørsholm, Denmark

Corresponding author
Michael Feigh - mfe@gubra.dk

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.

The present study aimed to evaluate prophylactic 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 3 weeks before treatment start (i.e. prior to onset 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 (SC, PO) 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) and fibrosis stage quantitative liver histology.

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

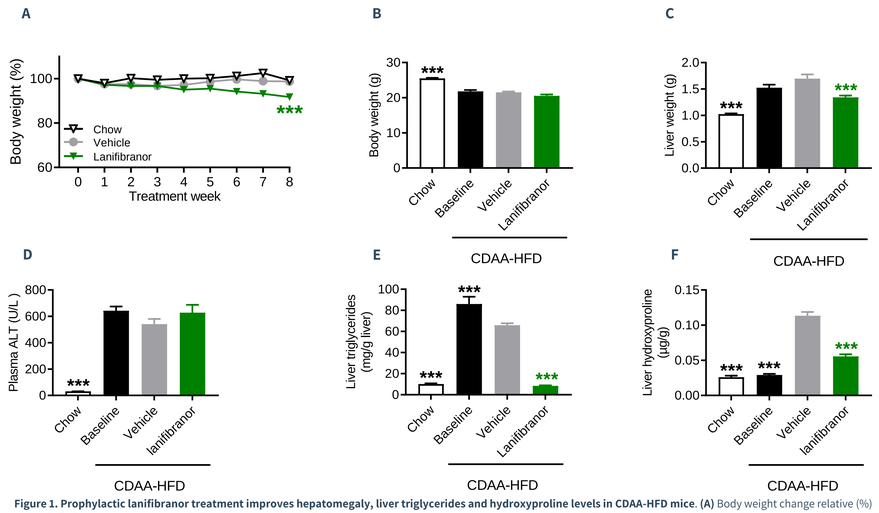

Figure 1. Prophylactic lanifibranor treatment improves hepatomegaly, liver triglycerides and hydroxyproline levels in CDAA-HFD mice. **(A)** Body weight change relative (%) to day 0 **(B)** Terminal body weight. **(C)** Terminal liver weight. **(D)** Terminal plasma alanine aminotransferase (ALT, U/L).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

3 NAFLD Activity Score and Fibrosis Stage

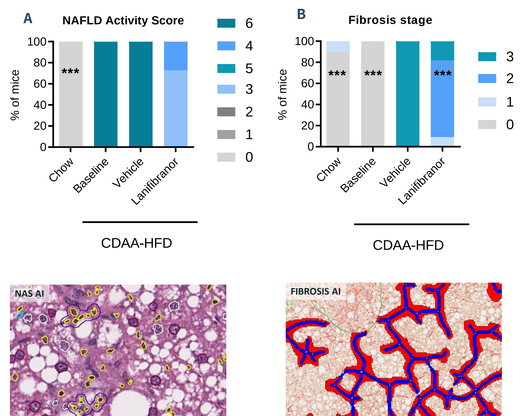

Figure 2. Prophylactic lanifibranor treatment improves NAFLD activity score and fibrosis stage in CDAA-HFD mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis 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

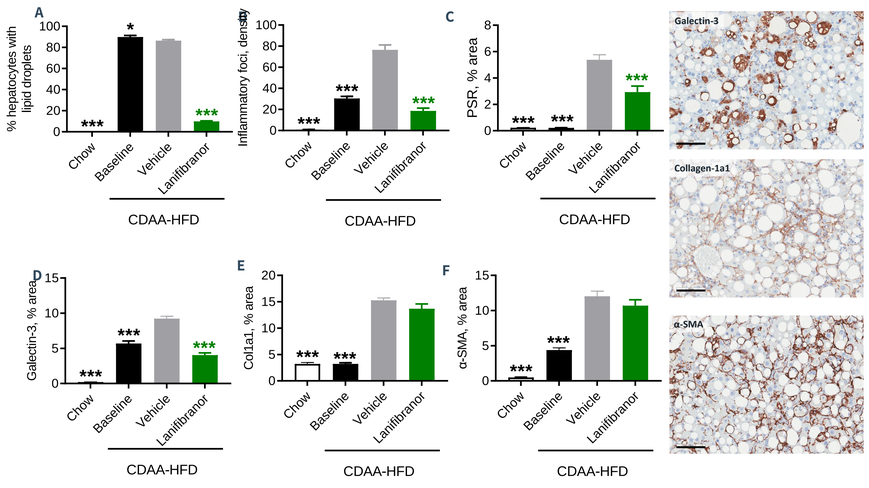

Figure 3. Prophylactic lanifibranor treatment improves quantitative histological markers of steatosis, inflammation and fibrogenesis in CDAA-HFD mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables and conventional IHC image analysis (**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 and plasma biochemistry

	Chow	Baseline	Vehicle	Lanifibranor
Plasma AST (U/L)	55.2 ± 2.7 ***	423 ± 26.4 *	566 ± 26	688 ± 70.9
Plasma ALT (U/L)	30.9 ± 0.5 ***	642 ± 32	541 ± 39	628 ± 58
Plasma TC (mmol/L)	2.2 ± 0.06 ***	2.23 ± 0.08 ***	1.55 ± 0.09	0.9 ± 0.08 ***
Plasma TG (mmol/L)	1.117 ± 0.09 ***	0.7 ± 0.03	0.7 ± 0.04	0.3 ± 0.02 ***
Plasma PIIINP (ng/mL)	0.5 ± 0.1 ***	5.36 ± 0.3 ***	13.2 ± 0.6	8.18 ± 0.5 ***
Plasma TIMP-1	623 ± 25.8 ***	2761 ± 127 ***	7047.6 ± 365	3 982 ± 224 ***
Liver TG (mg/g liver)	10.1 ± 0.7 ***	86 ± 7 **	66 ± 1.8	8.4 ± 0.6 ***
Liver TC (mg/g liver)	2.29 ± 0.1 ***	7.8 ± 0.9	7.16 ±0.4	2.14 ± 0.2 ***

Table 1. Prophylactic lanifibranor treatment improves plasma and liver biochemistry levels in CDAA-HFD mice. Abbreviations:, ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, total cholesterol, TG, total triglycerides; PIIINP, Type III procollagen peptide; TIMP-1, tissue inhibitor of metalloproteinase-1;. Mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 compared to corresponding CDAA-HFD vehicle group (Dunnett's test one-factor linear model).

CONCLUSION

Prophylactic lanifibranor treatment in CDAA-HFD mice:

+ Reduces hepatomegaly and liver hydroxyproline levels
+ Improves both NAFLD Activity Score and fibrosis stage
+ Reduces quantitative histological markers of steatosis, inflammation and fibrosis
+ Improves clinically relevant plasma and liver biochemical markers

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