Effect of Elafibranor treatment and dietary intervention in Gubra Amylin NASH (GAN) diet-induced obese mouse model of biopsy-confirmed non-alcoholic steatohepatitis

Michael Feigh[1*], Michelle L Boland[1], Henrik H Hansen[1], Eric Simon[2], Andre Broermann[2]

[1]Gubra, Hørsholm, Denmark; [2]Boehringer-Ingelheim Pharma GmbH & Co., Biberach an der Riss, Germany.
*Corresponding author: mfe@gubra.dk

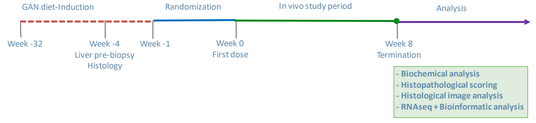
INTRODUCTION AND AIM

The trans-fat containing AMLN diet has been extensively validated in C57BL/6J mice for reliably inducing metabolic and hepatopathological changes recapitulating hallmarks of non-alcoholic steatohepatitis (NASH) with fibrosis. Due to a recent ban on trans-fats, we have recently introduced a trans-fat-free diet high in palm oil (Gubra Amylin NASH, GAN) with similar disease-inducing properties (World Journal of Gastroenterology, in press). Here, we characterized the therapeutic effects of elafibranor (PPAR-α/δ agonist) and dietary intervention in GAN diet-induced obese (DIO) NASH mice.

METHODS

Male C57BL/6J mice were fed GAN diet (40 kcal-% fat, 22% fructose, 10% sucrose, 2% cholesterol; D09100310, Research Diets) for 28 weeks prior to liver biopsy procedure. Only animals with biopsy-confirmed steatosis (score ≥2) and fibrosis (stage ≥F1) were included and stratified into treatment groups. GAN DIO-NASH mice received vehicle (PO, QD), elafibranor (30 mg/kg, PO, QD), or vehicle plus dietary intervention by shifting to chow feeding (chow-reversal), for 8 weeks. Vehicle-dosed chow-fed C57BL/6J mice served as normal controls (lean chow vehicle). Pre-post liver biopsy histopathological scoring was performed for within-subject evaluation of NAFLD Activity Score (NAS) and Fibrosis Stage. Terminal quantitative liver histology by morphometric image analysis, liver transcriptome analysis by RNAsequencing, blood and liver biochemistry were assessed.

STUDY DESIGN



Group #	Animal	Gender	Strain	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing volume	Dosing concentration
1	LEAN-CHOW	Male	C57BL/6JRj	10	Vehicle	PO	QD	5 ml/kg	-
2	DIO-NASH	Male	C57BL/6JRj	12	Vehicle	PO	QD	5 ml/kg	-
3	DIO-NASH	Male	C57BL/6JRj	12	Elafibranor	PO	QD	5 ml/kg	30 mg/kg
4	DIO-NASH	Male	C57BL/6JRj	12	Chow-reversal (vehicle)	PO	QD	5 ml/kg	-

RESULTS

Baseline characteristics in GAN DIO-NASH mice

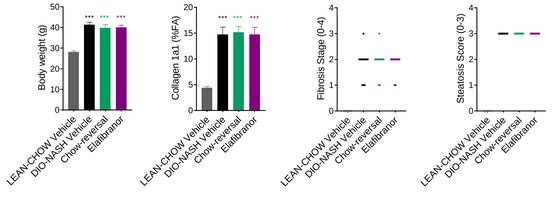


Figure 1 | Liver pre-biopsy stratification and randomization into study groups after 28 weeks on GAN diet. Inclusion criteria for GAN DIO-NASH mice were based on steatosis score (≥2), fibrosis stage (≥1) and randomization based on body weight and collagen 1a1 (% fractional area) by morphometric analysis.***p<0.001 vs. LEAN-CHOW Vehicle group.

Effect of Elafibranor and dietary intervention on metabolic and biochemical parameters in GAN DIO-NASH mice

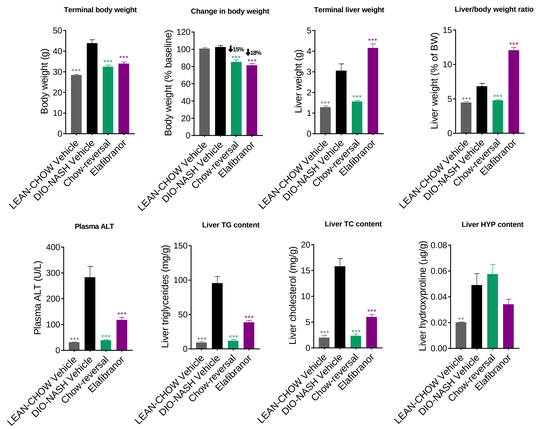


Figure 2 | Terminal body weight, liver weight and biochemical metabolic parameters. ALT, alanine transaminase; T, total triglycerides, TC, total cholesterol, HYP, hydroxyproline. **p<0.01, ***p<0.001 vs. DIO-NASH Vehicle.

Effect of Elafibranor and dietary intervention on histopathological morphometry in GAN DIO-NASH mice

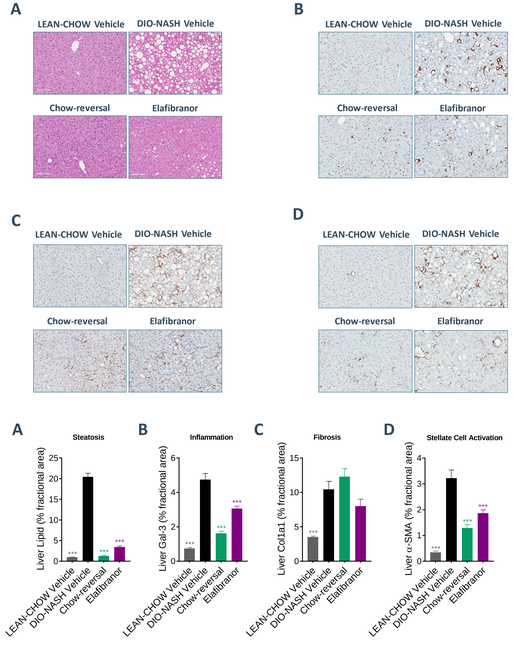


Figure 3 | Representative images and fractional area (%) determined by terminal liver histomorphometry for (A) steatosis (HE), (B) inflammation (Galectin-3), (C) fibrosis (Collagen 1a1) and (D) stellate cell activation (α-SMA). ***p<0.001 vs. DIO-NASH Vehicle group.

Effect of Elafibranor and dietary intervention on histopathological scoring in GAN DIO-NASH mice

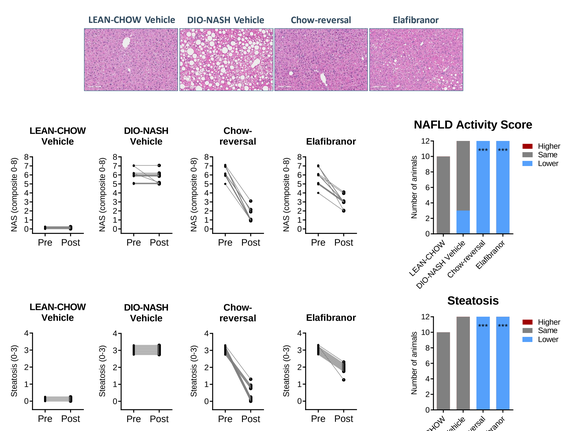


Figure 4 Summary of histopathological scoring of pre-and post-study liver biopsies for all animals separated by groups. For each intervention group, significance of number of animals with a lower score versus DIO-NASH vehicle was assessed using Fisher's exact test followed by adjustment for multiple correction using the Bonferroni method. **p<0.01, ***p<0.001 vs. DIO-NASH Vehicle group.

Effect of Elafibranor and dietary intervention on pro-fibrogenic gene markers in GAN DIO-NASH mice

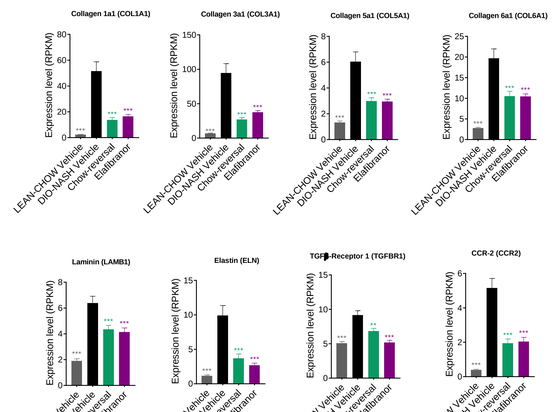


Figure 5 | Terminal liver transcriptomic profile for pro-fibrogenic gene markers. *p<0.05, **p<0.01, ***p<0.001 vs. DIO-NASH Vehicle group.

CONCLUSION

- The GAN DIO-NASH mouse model exhibits hallmark features of biopsy-confirmed fibrotic NASH and metabolic disease.
- Elafibranor and dietary intervention for 8 weeks in GAN DIO-NASH mice:
  - Improved the metabolic and biochemical profile
  - Reduced liver steatosis and inflammation by histomorphometry
  - Improved composite NAFLD Activity Score (pre-to-post)
  - Reduced hepatic stellate cell activation by histomorphometry
  - Reduced transcriptional pro-fibrogenic gene markers
- Longer treatment periods may be required to reverse liver fibrosis by histomorphometry and Fibrosis Stage (pre-to-post) in GAN DIO-NASH mice
- The GAN DIO-NASH mouse model demonstrates suitability for characterizing novel drug therapies for fibrotic NASH.