Metabolic, Biochemical, Histological, and Transcriptomic Effects of Combined Treatment with Semaglutide and Lanifibranor in the GAN Diet-Induced Obese and Biopsy-Confirmed Mouse Model of NASH

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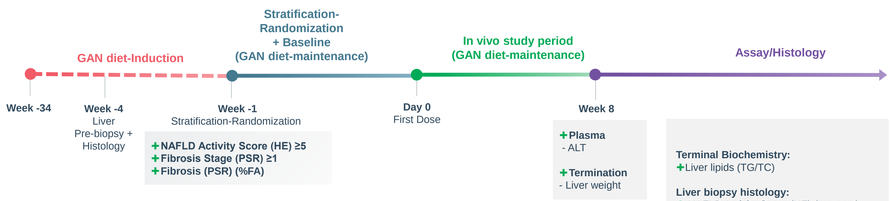
Background & Aim

Semaglutide (GLP-1 analogue) and lanifibranor (pan-PPAR agonist) have both showed promising therapeutic efficacy in recent phase 2 clinical trials for NASH. The present study aimed to evaluate the metabolic, biochemical, histopathological and transcriptomic effects of semaglutide and lanifibranor combination treatment using low doses, as compared to maximal dose monotherapy 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	**10**	**Vehicle**	SC + PO	QD + QD	5 ml/kg	-
2	DIO-NASH	Male	C57BL/6JRj	14	**Vehicle**	SC + PO	QD + QD	5 ml/kg	-
3	DIO-NASH	Male	C57BL/6JRj	14	**Semaglutide** + Vehicle	**SC** + PO	**QD** + QD	5 ml/kg	**30 nmol/kg**
4	DIO-NASH	Male	C57BL/6JRj	14	**Lanifibranor** + Vehicle	**PO** + SC	**QD** + QD	5 ml/kg	**30 mg/kg**
5	DIO-NASH	Male	C57BL/6JRj	14	**Lanifibranor** + **Semaglutide**	**PO** + SC	**QD** + QD	5 ml/kg	**10 mg/kg + 10 nmol/kg**

Terminal Biochemistry:
+Liver lipids (TG/TC)

Liver biopsy histology:
+ NAFLD Activity Score (HE) (pre-post)
+ Fibrosis Stage (PSR) (pre-post)
+ Terminal morphometric image analysis:
- Hepatocytes with lipids (HE)
- Inflammatory foci (HE)
- Inflammation (Gal-3) (IHC)
- Fibrosis (PSR)
- Collagen (Col1a1) (IHC)
- Stellate cell activation (α-SMA) (IHC)

Liver RNAseq and bioinformatic analysis

Improvement in metabolic and biochemical parameters

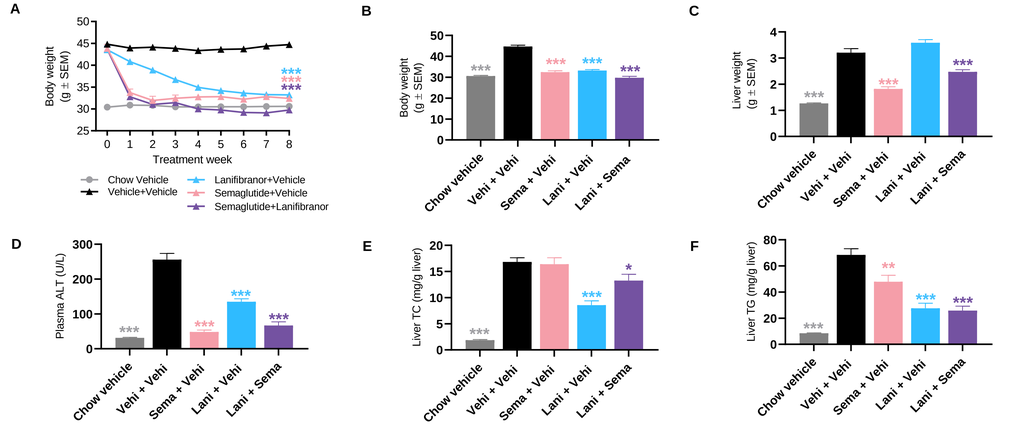


Figure 1. Semaglutide and lanifibranor combination improves metabolic and biochemical parameters in GAN DIO-NASH mice. **(A)** Body weight change during treatment period. **(B)** Terminal body weight. **(C)** Terminal liver weight. **(D)** Terminal plasma alanine aminotransferase (ALT). **(E)** Terminal liver total cholesterol (TC). **(F)** Terminal liver triglycerides (TG). *p<0.05, **p<0.01, ***p<0.001 compared to corresponding vehicle + vehicle control (Dunnett's test one-factor linear model).

Improvement in NAFLD Activity Score

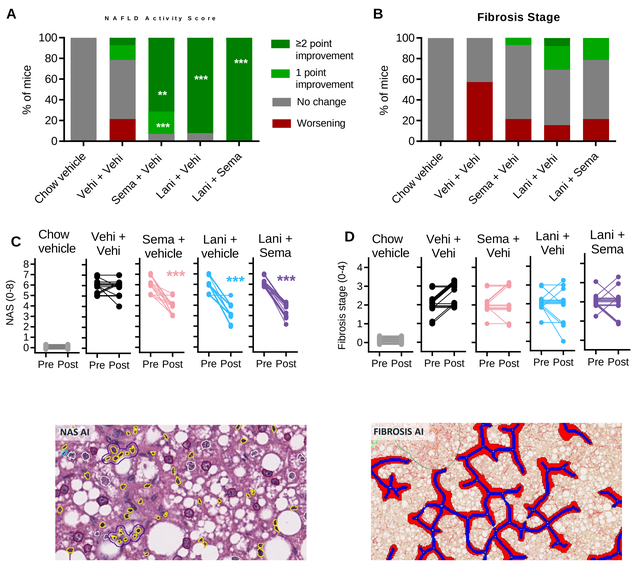


Figure 2. Semaglutide and lanifibranor combination improves NAFLD Activity Score in GAN DIO-NASH mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis stage. **(C)** Comparison of individual pre-post NAS. **(D)** Comparison of individual pre-post Fibrosis stage. **p<0.01, ***p<0.001 to corresponding vehicle + 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

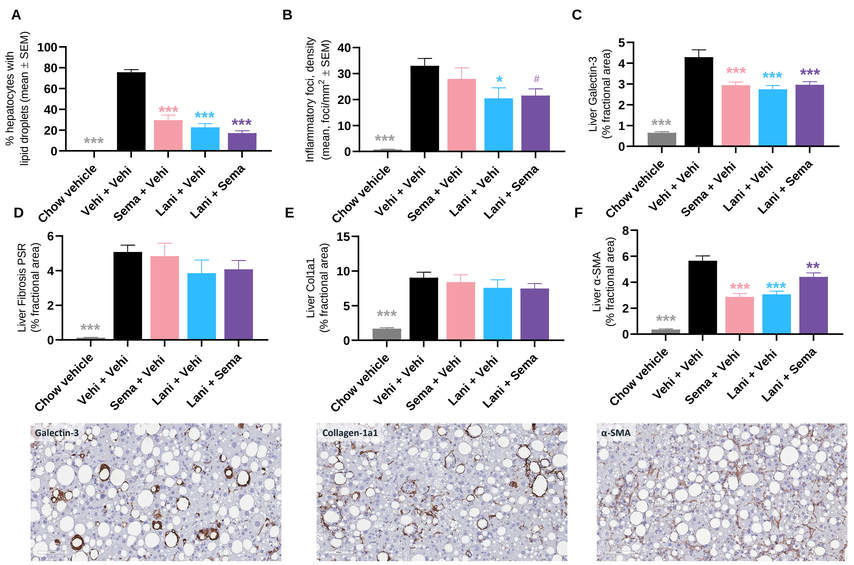


Figure 3. Semaglutide and lanifibranor combination improves quantitative liver histological markers in GAN DIO-NASH mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). **(A)** % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of galectin-3. **(D)** % area of PSR. **(E)** % area of collagen-1a1 (Col1a1). **(F)** % area of alpha-smooth muscle actin (α-SMA). Mean ± SEM. *p<0.05, ***p<0.001, #p=0.059 to corresponding vehicle + vehicle group (Dunnett's test one-factor linear model). Bottom panels: Representative galectin-3, collagen 1a1 and α-SMA photomicrographs (scale bar, 100 µm).

Improvement in transcriptomic profile for fibrosis

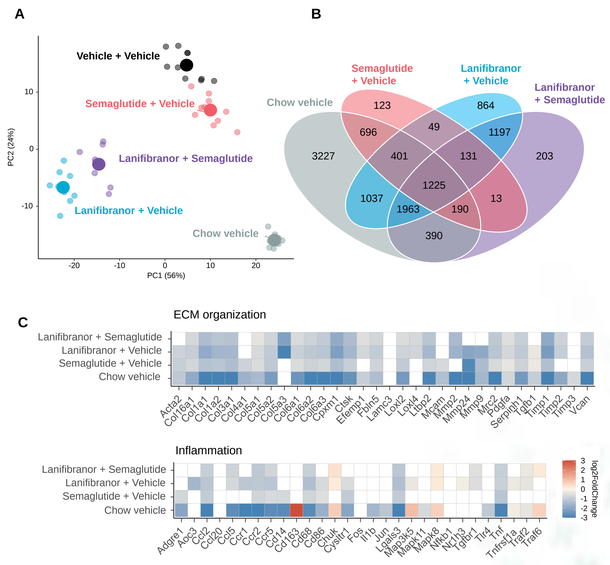


Figure 4. Semaglutide and lanifibranor combination suppress fibrosis-associated genes in GAN DIO-NASH mice. **(A)** Principal component analysis (PCA) of samples based on top 500 most variable gene expression levels. **(B)** Venn diagram depicting shared and separate differentially expressed genes in treatment groups. **(C)** Regulation of hepatic extracellular matrix (ECM) and inflammation candidate genes. Blue and red colour gradients indicate significantly (p<0.05) down-regulated and up-regulated gene expression, respectively. White boxes indicate genes not regulated (p>0.05) compared to vehicle + vehicle mice.

CONCLUSION

+ Combined low-dose semaglutide and lanifibranor treatment demonstrate similar therapeutic effects as compared to high-dose monotherapy:
  - Reduction in body and liver weight
  - Reduction in plasma ALT and liver lipids.
  - ≥2-point significant improvement in NAFLD Activity Score.
  - Reduction in quantitative histological markers of steatosis, inflammation and stellate cell activation.
  - Suppression in fibrosis-associated genes.
+ These findings highlight the use of the GAN DIO-NASH mouse model for exploring novel combinatorial therapies for the treatment of NASH.