



Semaglutide, but not lanifibranor, promotes tumor regression in the GAN diet-induced obese and biopsy-confirmed mouse model of NASH with advanced fibrosis and HCC



Mathias Bonde Møllerhøj¹, Mogens Vyberg², Henrik H. Hansen¹, Michael Feigh¹

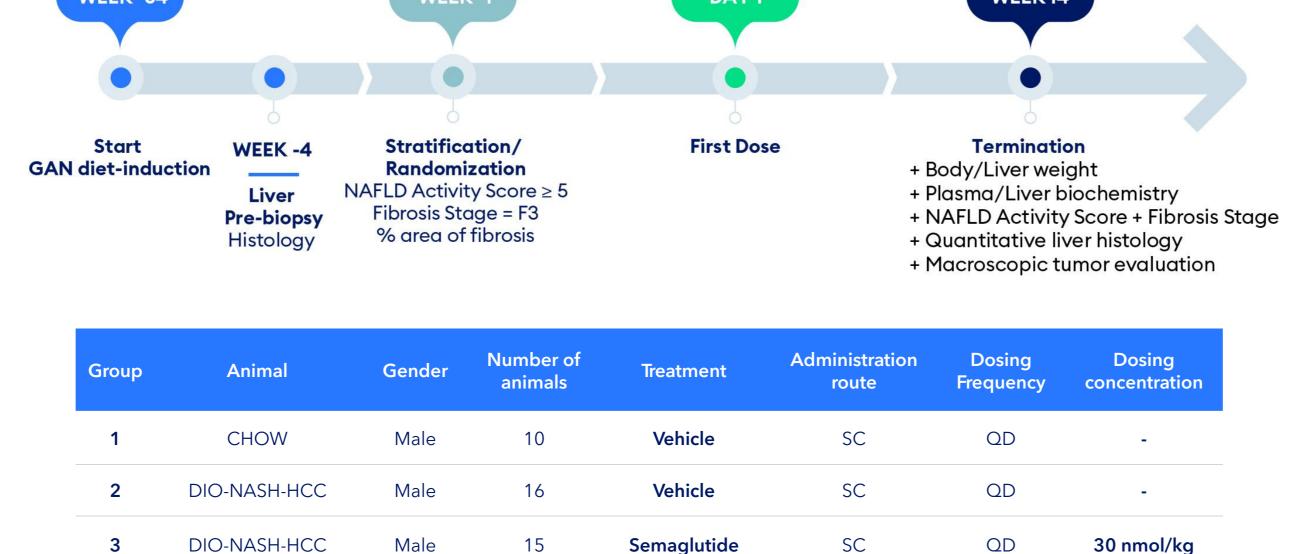
¹ Gubra, Hørsholm Kongevej 11B, Hørsholm, Denmark

² Center for RNA Medicine, Department of Clinical Medicine, Aalborg University, Copenhagen, Denmark

Corresponding author Denise Oró - dor@gubra.dk

Background & Aim

Non-alcoholic steatohepatitis (NASH) is emerging as a major cause of hepatocellular carcinoma (HCC). Semaglutide (glucagon like petide-1 (GLP-1) receptor agonist) and lanifibranor (pan peroxisome proliferator activated receptor (pan-PPAR) agonist) are currently in late-stage clinical development for NASH. The present study aimed to evaluate the efficacy of semaglutide and lanifibranor monotherapy on disease progression in the translational Gubra Amylin NASH (GAN) diet-induced obese (DIO) and biopsy-confirmed mouse model of advanced fibrosing NASH and HCC.



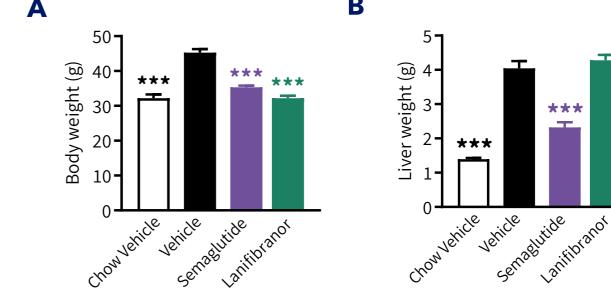


Figure 2. Semaglutide and lanibranor improves body weight and plasma transaminases in GAN DIO-NASH-HCC mice. (A) Terminal body weight (g). (B) Terminal liver weight (g). (C) Terminal plasma alanine transaminase (ALT, U/L). (D) Terminal plasma aspartate aminotransferase (AST, U/L). ***p<0.001 compared to DIO-NASH-HCC vehicle group (Dunnett's test one-factor linear model).



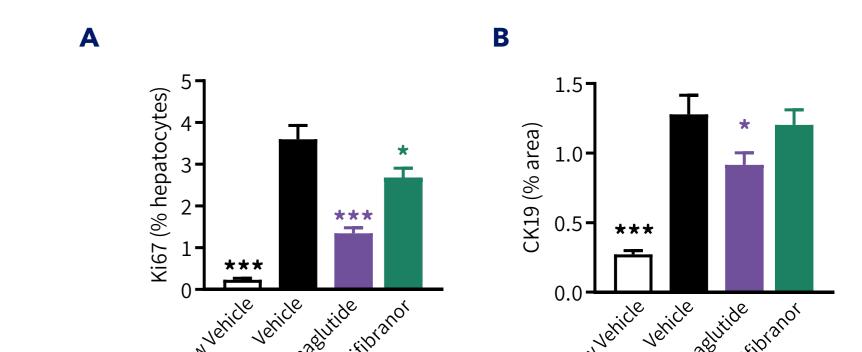
С

ALT (U/L) 500: 500:

200-

100-

Histological markers of proliferation and progenitor cell activation



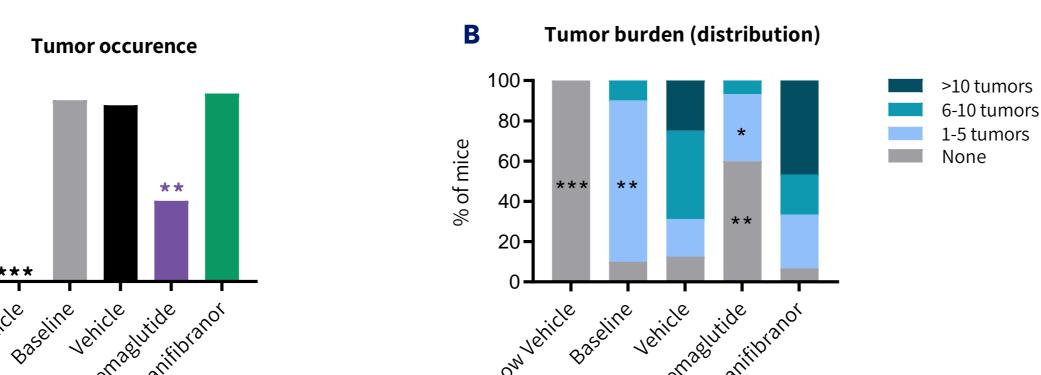
Hepatocellular carcinoma occurrence and burden

Lanifibranor

PO

QD

30 mg/kg



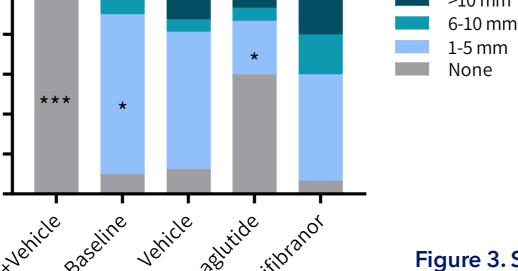
Methods

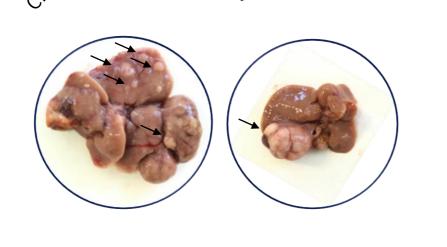
Male C57BL/6J mice were fed the GAN diet high in fat, fructose, and cholesterol for 54 weeks prior to treatment intervention. Only animals with liver biopsy-confirmed NAFLD Activity Score (NAS ≥5) and advanced fibrosis (stage F3) were included and stratified into study groups. DIO-NASH-HCC mice received vehicle, semaglutide, or lanifibranor for 14 weeks. Vehicledosed chow-fed C57BL/6J mice served as lean healthy controls. Untreated DIO-NASH-HCC mice (n=10) were terminated at baseline.

Conclusion

Both semaglutide and lanifibranor promotes ≥2-point improvement in NAFLD Activity Score

Largest tumor size (distribution)





DIO-NASH-HCC

3

100.

80.

60

40

20

(%)

JOL

with

Mice

Male

15

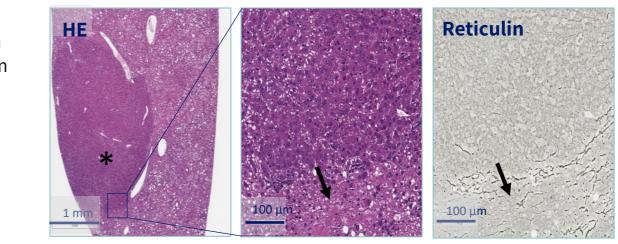
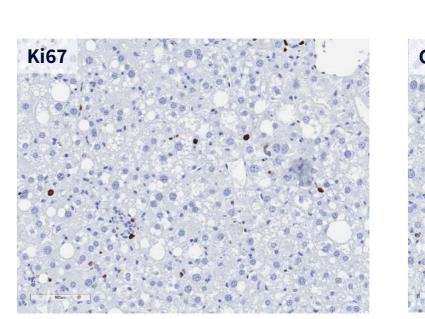


Figure 3. Semaglutide, but not lanifibranor, prevents HCC progression in GAN DIO-NASH-HCC mice. (A) Macroscopic (surface) tumor occurrence. B) Tumors numbers per animal. (C) Largest tumor size. (D) Representative images of HE and reticulin stained tumor sections. High resolution image demonstrating increased hepatocyte nuclear/cytoplasmic ratio (condensed cytoplasm with normal or enlarged nuclei) and absent reticulin trabecular framework. Asterisk marks a large tumor and arrows indicate the compression zone between the neoplastic and normal liver parenchyma. (E) Representative photos of macroscopic tumor burden in GAN DIO-NASH-HCC mice. *p<0.05, **p<0.01, ***p<0.001 compared to DIO-NASH-HCC vehicle group (Dunnett's test one-factor linear model).



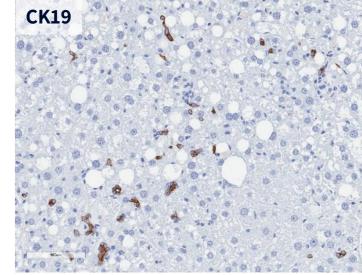
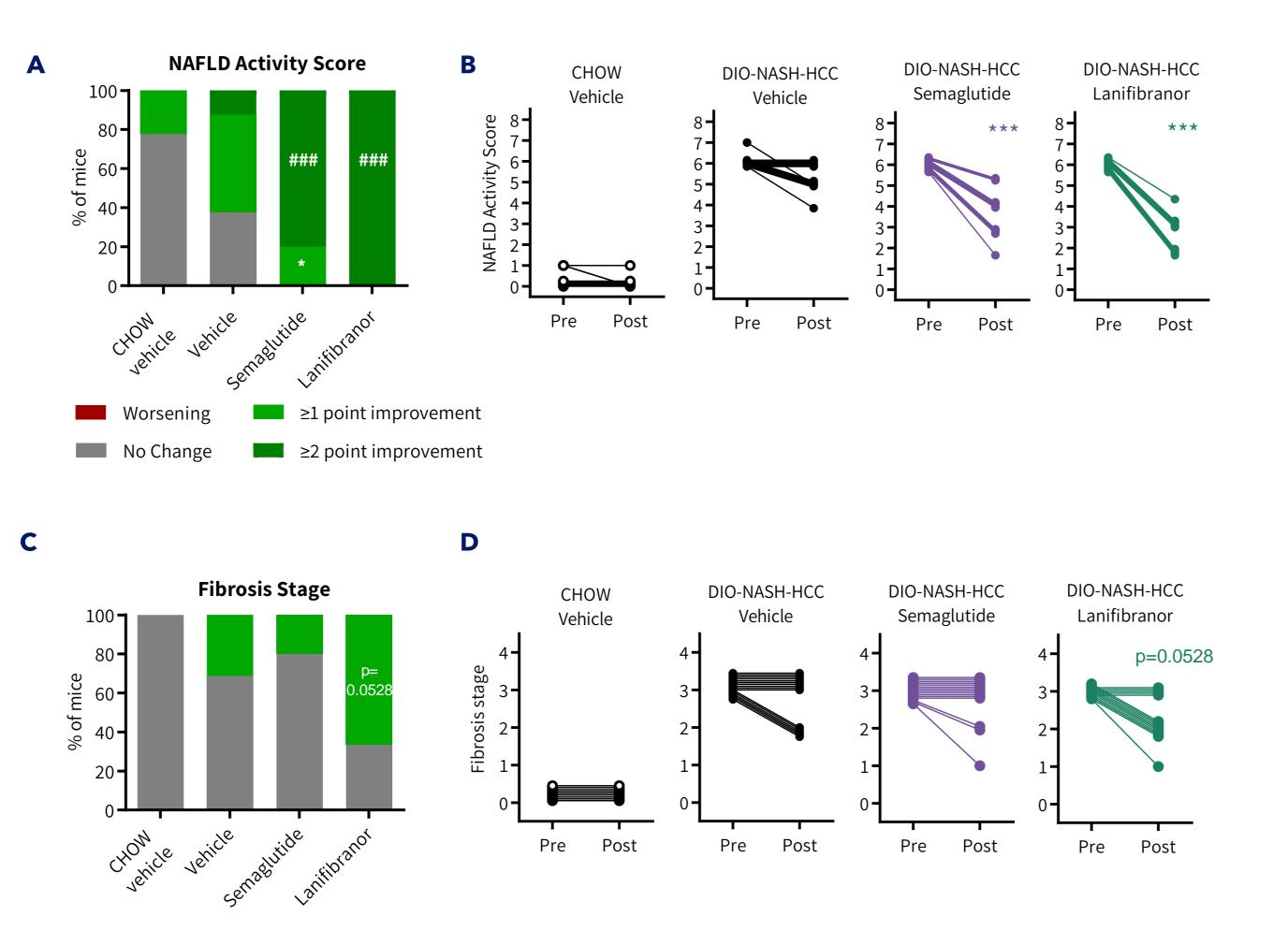
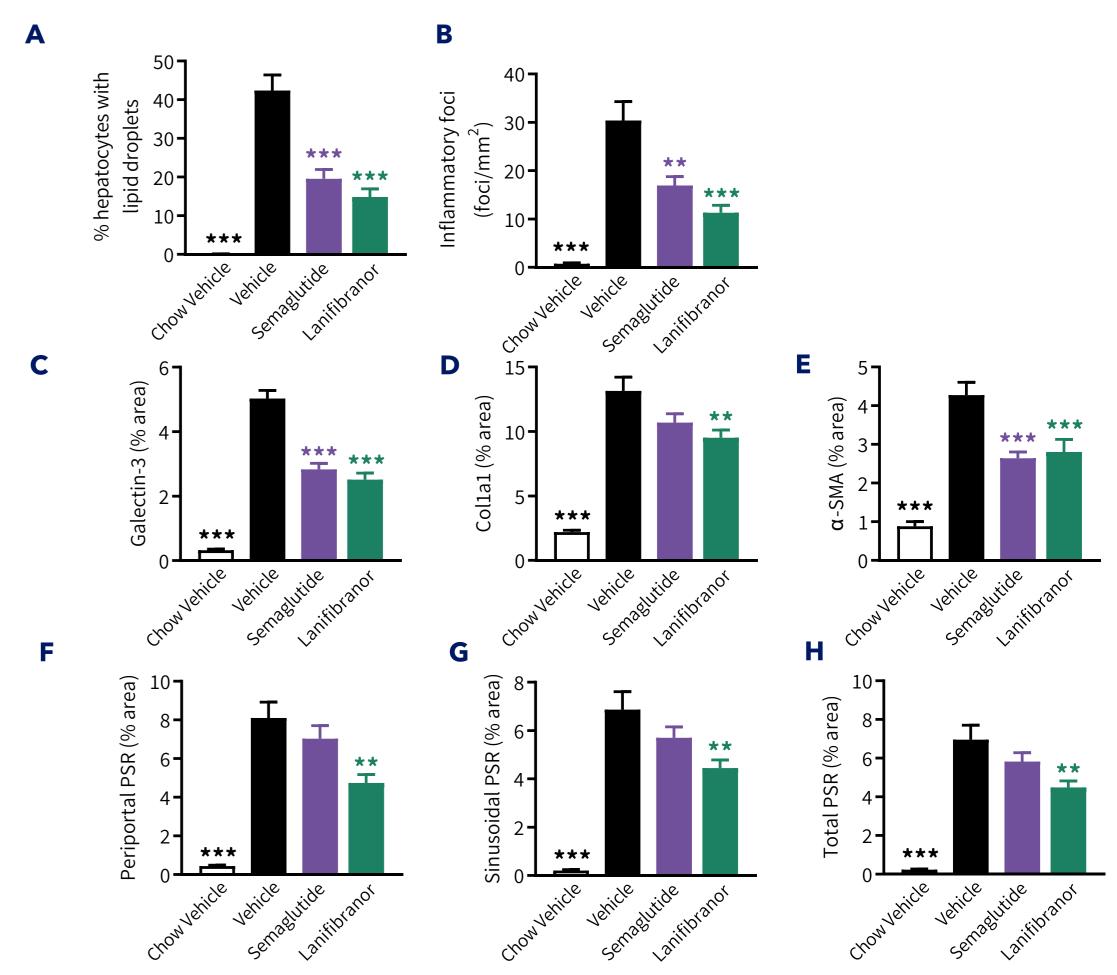


Figure 4. Semaglutide improves quantitative histological markers of proliferation and progenitor cells in GAN DIO-NASH-HCC mice. (A) % of Ki67-positive hepatocytes. (B) % area of CK19 staining. Mean ± SEM. (C) Representative Ki67 and CK19 photomicrographs (scale bar, 100 µm). *p<0.05, ***p<0.001 vs. DIO-NASH-HCC vehicle group (Dunnett's test one-factor linear model).

NAFLD Activity Score and Fibrosis Stage 5



Histological markers of steatosis, inflammation and fibrosis



- Lanifibranor improves fibrosis + histology
- Semaglutide reduces HCC burden
- The GAN DIO-NASH-HCC mouse is highly applicable for profiling novel drug therapies targeting NASH with advanced fibrosis and HCC







Figure 5. Semaglutide and lanifibranor improves NAFLD Activity Score in GAN DIO-NASH-HCC

mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. (A) NAFLD Activity Score (NAS). (B) Comparison of individual pre-post NAS. (C) Fibrosis stage (D) Comparison of individual pre-post Fibrosis Stage. *p<0.05 with one-point improvement, ###p<0.001 with more than 2-point improvement compared to corresponding DIO-NASH-HCC vehicle group (One-sided Fisher's exact test with Bonferroni correction). ***p<0.001 to DIO-NASH-HCC vehicle group.

Figure 6. Semaglutide and lanifibranor improves quantitative histological markers of steatosis, inflammation and fibrogenesis in GAN DIO-NASH-HCC 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 collagen-1a1. (E) % area of alpha-smooth muscle actin (α -SMA). (F-H) % area of PSR. Mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 to DIO-NASH-HCC vehicle group (Dunnett's test one-factor linear model).