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

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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.

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. Vehicle-dosed 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
+ 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

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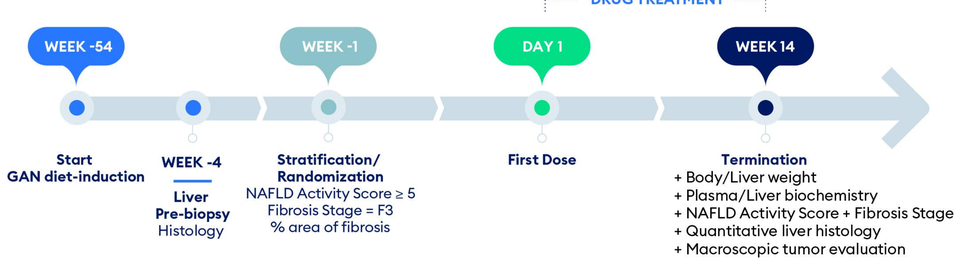
1 Study outline



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing concentration
1	CHOW	Male	10	**Vehicle**	SC	QD	-
2	DIO-NASH-HCC	Male	16	**Vehicle**	SC	QD	-
3	DIO-NASH-HCC	Male	15	**Semaglutide**	SC	QD	**30 nmol/kg**
4	DIO-NASH-HCC	Male	15	**Lanifibranor**	PO	QD	**30 mg/kg**

2 Metabolic and biochemical parameters

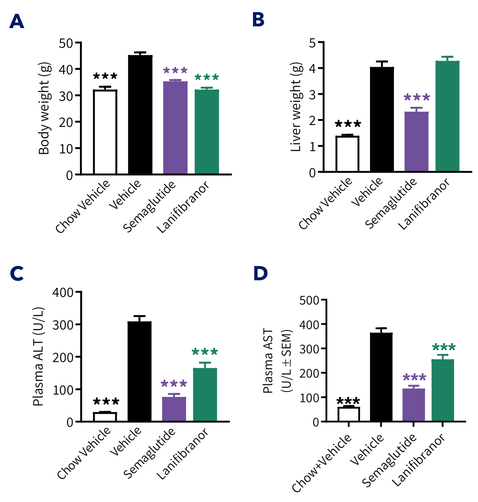


Figure 2. Semaglutide and lanibranor improves body weight and plasma transaminases in GAN DIO-NASH-HCC mice. (A) Terminal body weight (g). **(B)** Terminal liver weight (g). **(C)** Terminal plasma alanine transaminase (ALT, U/L). **(D)** Terminal plasma aspartate aminotransferase (AST, U/L). ***p<0.001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

3 Hepatocellular carcinoma occurrence and burden

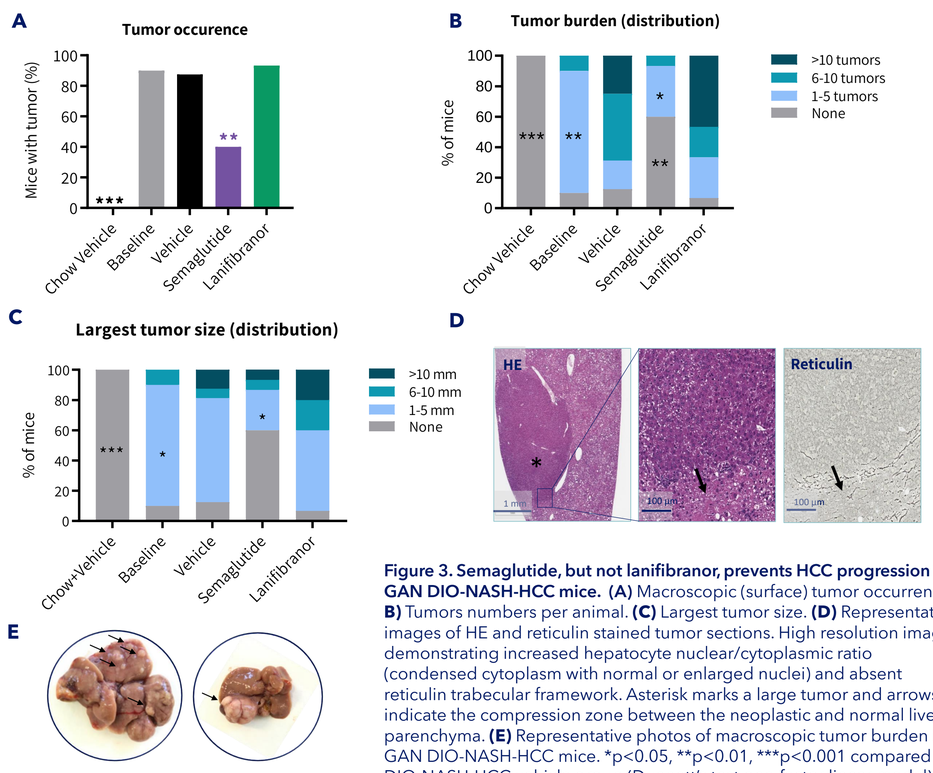
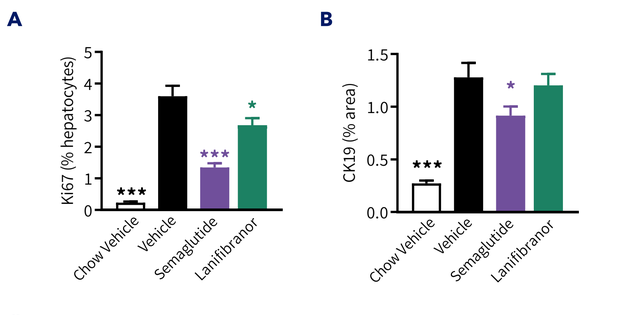


Figure 3. Semaglutide, but not lanifibranor, prevents HCC progression in GAN DIO-NASH-HCC mice. (A) Macroscopic (surface) tumor occurrence. **B)** Tumors numbers per animal. **(C)** Largest tumor size. **(D)** Representative images of HE and reticulin stained tumor sections. High resolution image demonstrating 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).

4 Histological markers of proliferation and progenitor cell activation



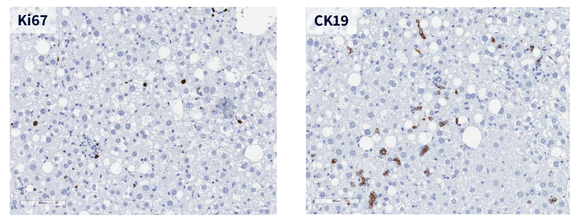


Figure 4. Semaglutide improves quantitative histological markers of proliferation and progenitor cells in GAN DIO-NASH-HCC mice. (A) % of Ki67-positive hepatocytes. **(B)** % area of CK19 staining. Mean ± SEM. **(C)** Representative Ki67 and CK19 photomicrographs (scale bar, 100 µm). *p<0.05, ***p<0.001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

5 NAFLD Activity Score and Fibrosis Stage

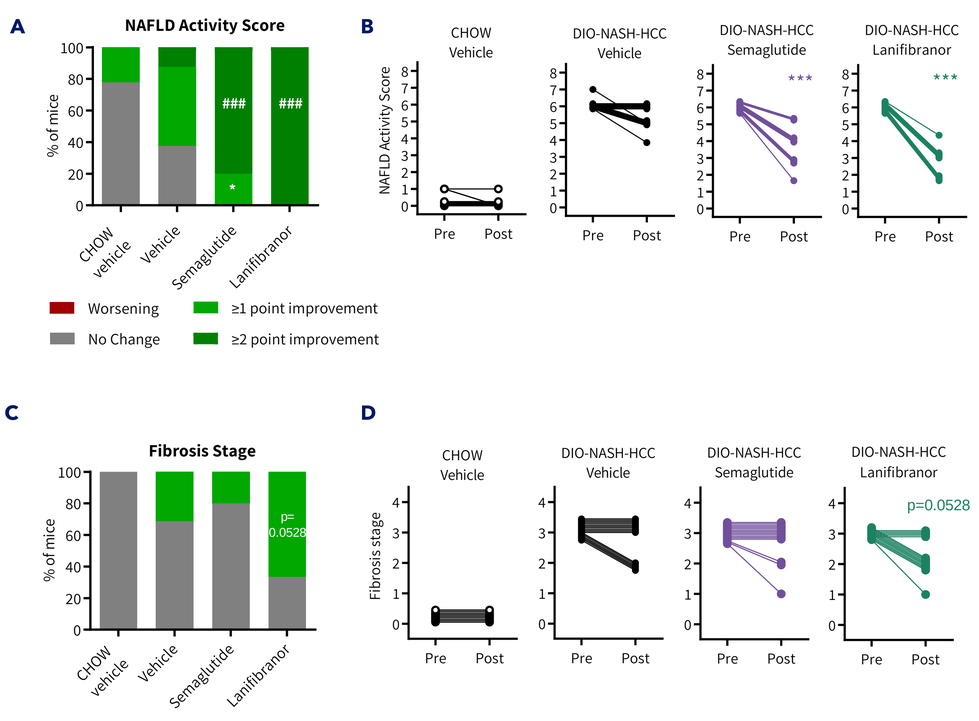


Figure 5. Semaglutide and lanifibranor improves NAFLD Activity Score in GAN DIO-NASH-HCC mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Comparison of individual 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.

6 Histological markers of steatosis, inflammation and fibrosis

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).