Semaglutide exerts anti-tumor action 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) increases the risk for the development of liver fibrosis which may progress to cirrhosis and hepatocellular carcinoma (HCC). Semaglutide (glucagon-like-receptor (GLP)-1 agonist) is currently in late-stage clinical development for NASH. The present study aimed to evaluate the hepatoprotective effects of semaglutide therapy in the 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. 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 (SC, QD, n=16), or semaglutide (SC, QD, 30 nmol/kg, n=15) for 14 weeks. Vehicle-dosed chow-fed C57BL/6J mice (SC, QD, n=10) served as lean healthy controls. Tumor histopathological evaluation was performed by a clinical histopathologist. Within-subject (pre-to-post) change in nonalcoholic fatty liver disease (NAFLD) Activity Score (NAS) and Fibrosis Stage was evaluated by Gubra Histopathological Objective Scoring Technique (GHOST). Other endpoints included terminal blood biochemistry and quantitative histomorphometry.

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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**

2 Metabolic and biochemical parameters

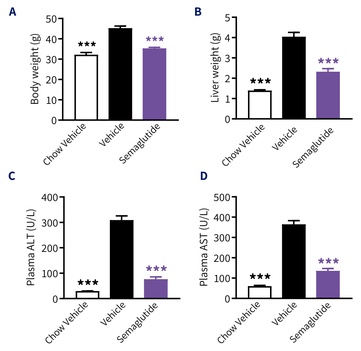
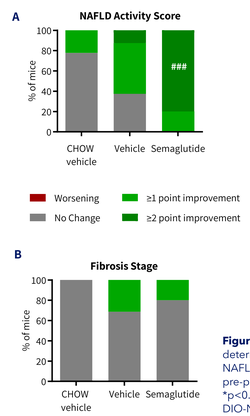


Figure 1. Semaglutide improves body weight and plasma biochemistry in GAN DIO-NASH-HCC mice. **(A)** Terminal body weight (g). **(B)** Terminal liver weight (g). **(C)** Terminal plasma alanine transaminase (ALT, U/L). **(D)** Terminal plasma aspartate aminotransferase (AST, U/L). ***p<0.001 compared to DIO-NASH-HCC vehicle group (Dunnett's test one-factor linear model).

3 NAFLD Activity Score and Fibrosis Stage



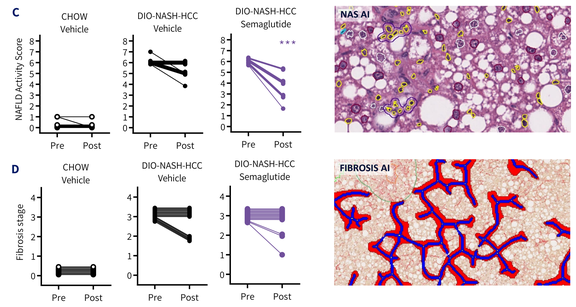


Figure 2. Semaglutide improves NAFLD Activity Score in GAN DIO-NASH-HCC mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis stage. **(C)** Comparison of individual pre-post NAS. **(D)** Comparison of individual pre-post Fibrosis Stage. Right panels: Representative HE and PSR photomicrographs used for GHOST evaluation. *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).

4 Histological markers of steatosis, inflammation and fibrosis

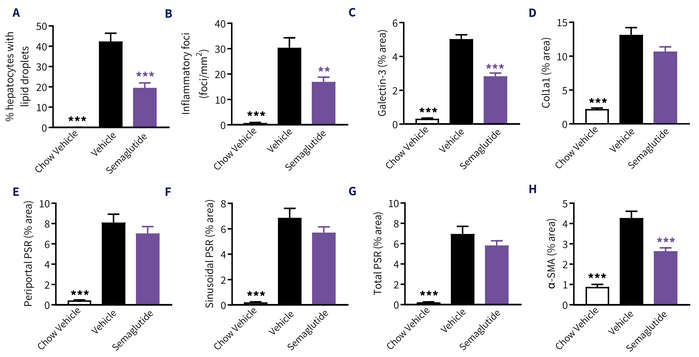


Figure 3. Semaglutide improves quantitative histological markers of steatosis, inflammation and fibrogenesis in GAN DIO-NASH-HCC mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). **(A)** % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of galectin-3. **D)** % area of collagen-1a1. **(E-G)** % area of PSR. **(H)** % area of alpha-smooth muscle actin (α-SMA). Bottom panels: Representative galectin-3, collagen 1a1 and α-SMA photomicrographs for semaglutide treatment group (scale bar, 100 µm). Mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 to DIO-NASH-HCC vehicle group (Dunnett's test one-factor linear model).

5 Hepatocellular carcinoma occurrence and burden

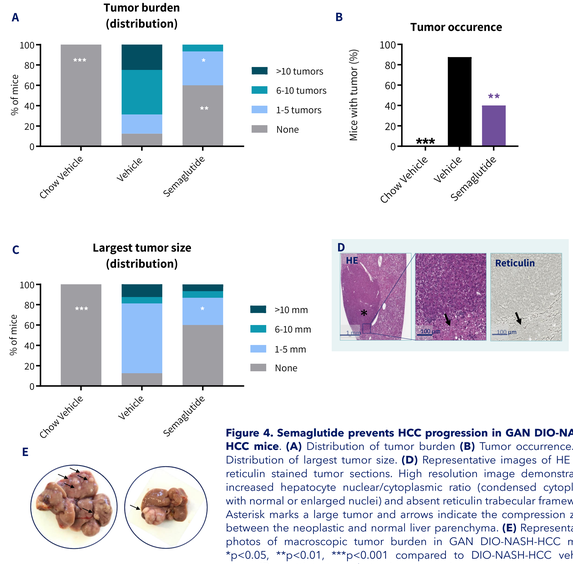
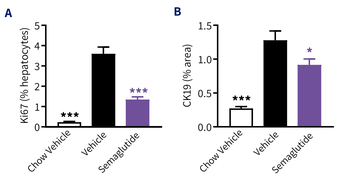


Figure 4. Semaglutide prevents HCC progression in GAN DIO-NASH-HCC mice. (A) Distribution of tumor burden **(B)** Tumor occurrence. **(C)** Distribution of largest tumor size. **(D)** Representative images of HE and reticulin stained tumor sections. High resolution image demonstrating increased hepatocyte 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).

6 Histological markers of proliferation and progenitor cell activation



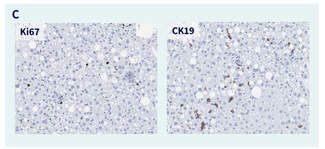


Figure 5. Semaglutide improves quantitative histological markers of proliferation and progenitor cells in GAN DIO-NASH-HCC mice. (A) % of Ki67-positive hepatocytes. **(B)** % area of CK19 staining. Mean ± SEM. **(C)** Representative Ki67 and CK19 photomicrographs (scale bar, 100 µm). *p<0.05, ***p<0.001 vs. DIO-NASH-HCC vehicle group (Dunnett's test one-factor linear model).

Conclusion

- Semaglutide reduces body weight in GAN DIO-NASH-HCC mice
- Semaglutide improves hepatomegaly, and improved plasma biochemistry
- Semaglutide promotes ≥2-point significant improvement in NAFLD Activity Score
- Semaglutide does not improve fibrosis stage and quantitative fibrosis histology
- Semaglutide shows beneficial effects on quantitative histological markers of steatosis, inflammation and fibrogenesis
- Semaglutide markedly reduces HCC burden
- The GAN DIO-NASH-HCC mouse model is highly applicable for profiling novel drug therapies targeting NASH with advanced fibrosis and HCC

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