Semaglutide exerts anti-tumor action in the GAN diet-induced obese and biopsy-confirmed mouse model of MASH with advanced fibrosis and HCC

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Background & Aim

metabolic dysfunction-associated steatohepatitis (MASH) 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 MASH. 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 MASH and HCC.

Method

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-MASH-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 classification was performed by a clinical histopathologist. Within-subject (pre-to-post) change in nonalcoholic fatty liver disease (NAFLD) Activity Score (NAS), fibrosis stage, and collagen deposition (PSR % area) was evaluated Other endpoints included terminal blood biochemistry and quantitative histomorphometry.

Conclusion

+ Semaglutide reduces body weight in GAN DIO-MASH-HCC mice
+ Semaglutide improves hepatomegaly, and improved plasma transaminases
+ 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-MASH-HCC mouse model is highly applicable for profiling novel drug therapies targeting MASH 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-MASH-HCC	Male	16	**Vehicle**	SC	QD	-
3	DIO-MASH-HCC	Male	15	**Semaglutide**	SC	QD	**30 nmol/kg**

2 Metabolic and biochemical parameters

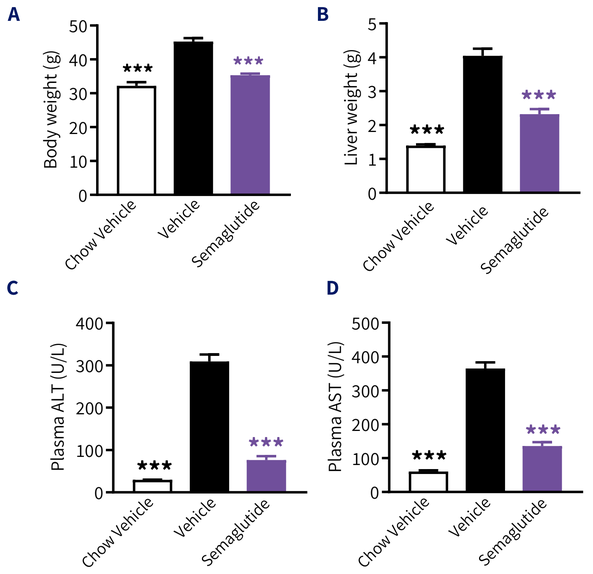

Figure 1. Semaglutide improves body weight and plasma transaminases in GAN DIO-MASH-HCC mice. **(A)** Terminal body weight (g). **(B)** Terminal liver weight (g). **(C)** Terminal plasma alanine transaminase (ALT, U/L). **(D)** Terminal plasma aspartate aminotransferase (AST, U/L). ***p<0.001 compared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

3 NAFLD activity score and fibrosis stage

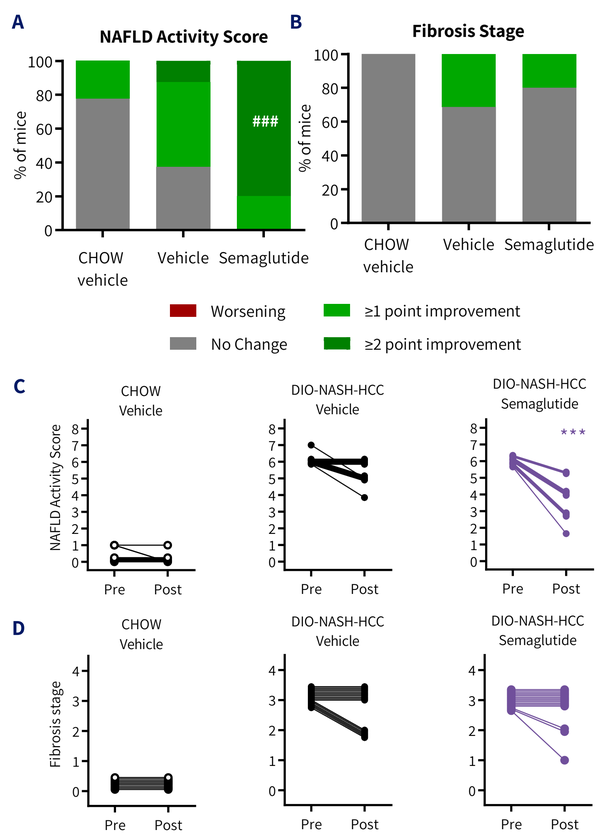

Figure 2. Semaglutide improves NAFLD Activity Score in GAN DIO-MASH-HCC mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis stage. **(C)** Comparison of individual pre-post NAS. **(D)** Right panels: Representative HE and PSR photomicrographs used for GHOST evaluation. 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-MASH-HCC vehicle group (One-sided Fisher's exact test with Bonferroni correction).

4 Histological markers of steatosis, inflammation and fibrosis

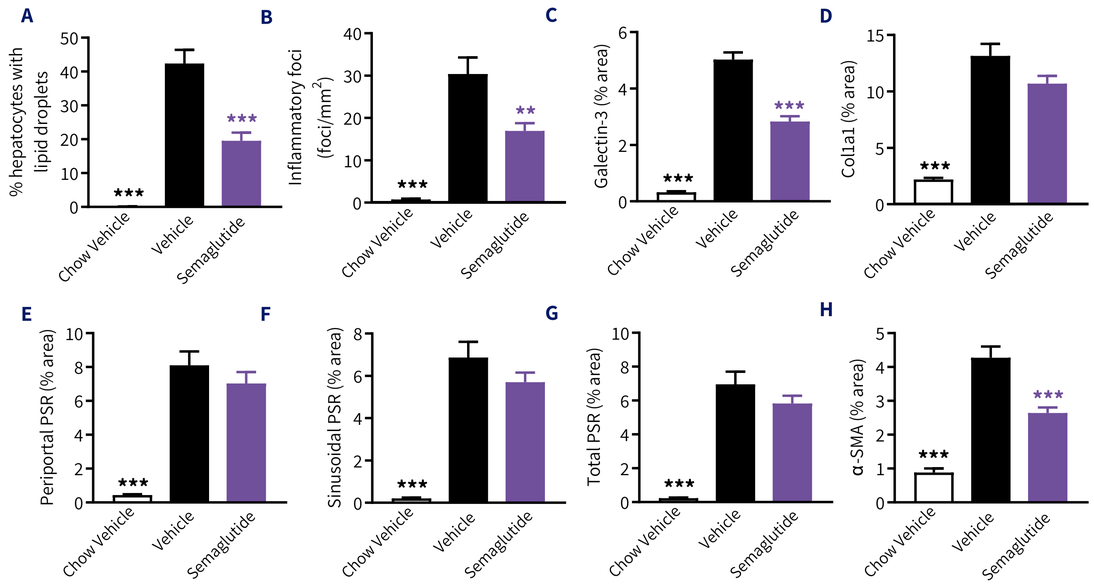

Figure 3. Semaglutide improves quantitative histological markers of steatosis, inflammation and fibrogenesis in GAN DIO-MASH-HCC mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (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). Mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 to DIO-MASH-HCC vehicle group (Dunnett's test one-factor linear model). Bottom panels: Representative galectin-3, collagen 1a1 and α-SMA photomicrographs for semaglutide treatment group (scale bar, 100 µm).

5 Hepatocellular carcinoma occurrence and burden

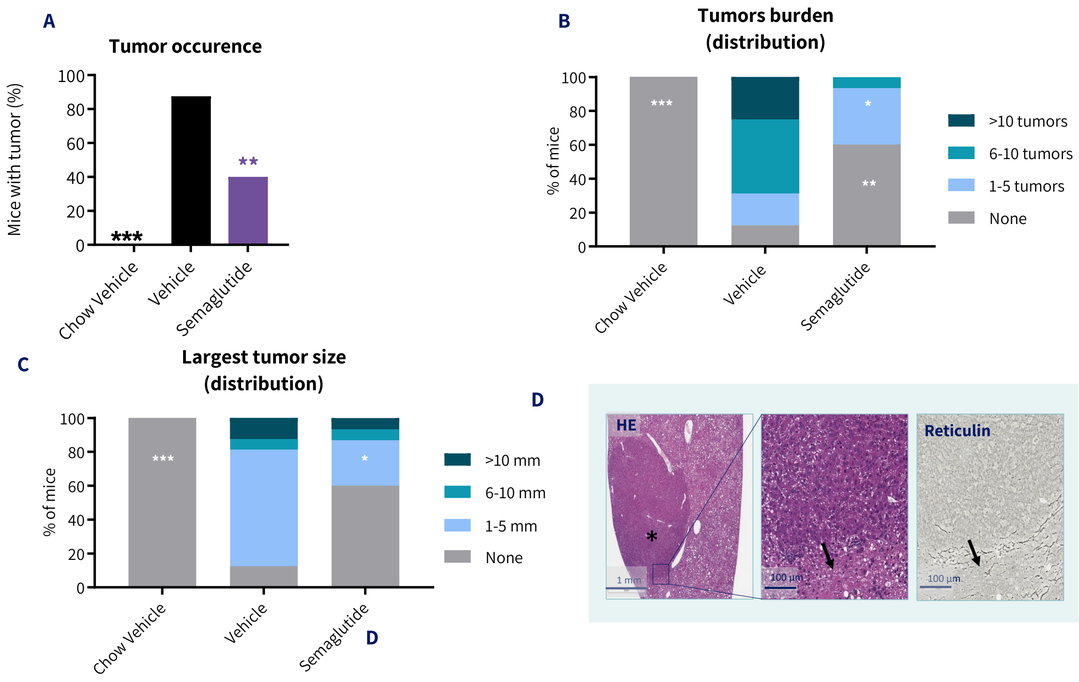

Figure 4. Semaglutide prevents HCC progression in GAN DIO-MASH-HCC mice. **(A)** Macroscopic (surface) tumor occurrence **(B)** Tumors numbers per animal. **(C)** Largest tumor size. **(D)** Representative images of HE and reticulin stained tumor sections. High resolution image demonstrating increased 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-MASH-HCC mice. *p<0.05, **p<0.01, ***p<0.001 compared to DIO-MASH-HCC vehicle group (Dunnett's test one-factor linear model).

6 Histological markers of proliferation and progenitor cell activation

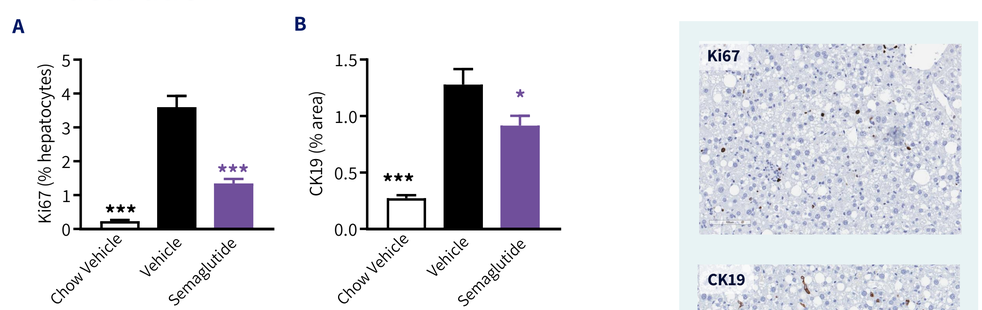

Figure 5. Semaglutide improves quantitative histological markers of proliferation and progenitor cells in GAN DIO-MASH-HCC mice. (A) % of Ki67-positive hepatocytes. **(B)** % area of CK19 staining. Mean ± SEM. **(C)** Representative Ki67 and CK19 photomicrographs (scale bar, 100 µm). *p<0.05, ***p<0.001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

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