Tirzepatide reduces tumor burden in the GAN diet-induced obese and biopsy-confirmed mouse model of MASH-HCC with advanced fibrosis

Authors
Michael Feigh[1], Susanne E. Pors[1], Jacob Nøhr-Meldgaard[1], Nicolas Eskesen[1], Henrik H. Hansen[1],

[1] Gubra, Hørsholm Kongevej 11B, Hørsholm, Denmark

Corresponding author
Michael Feigh- **mfe@gubra.dk**

Background & Aim

The glucagon-like peptide-1 receptor (GLP1R) and gastric inhibitory polypeptide receptor (GIPR) dual agonist tirzepatide is currently approved for the treatment of obesity and type 2 diabetes.

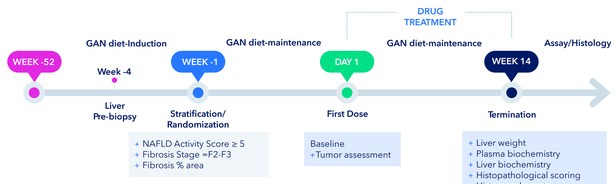
In a recent phase 2b trial (SYNERGY-NASH) in patients with metabolic-dysfunction associated steatohepatitis (MASH), tirzepatide showed a significant effect on MASH resolution without worsening fibrosis after 52 weeks (Loomba et al., NEJM, 2024).

The present study aimed to evaluate therapeutic efficacy of tirzepatide on clinically relevant metabolic, biochemical and histopathological outcomes in addition to tumor burden in the GAN diet-induced obese (DIO) mouse model of MASH with advanced fibrosis and hepatocellular carcinoma (HCC).

Method

Male C57BL/6J mice were fed the GAN diet high in fat, fructose, and cholesterol for 52 weeks prior to treatment intervention. Animals with liver biopsy-confirmed NAFLD Activity Score (NAS ≥5) and advanced fibrosis (stage F2-F3) were included and stratified into study groups. DIO-MASH-HCC mice received vehicle (SC, QD), or Tirzepatide (SC, QD, 30 nmol/kg) for 14 weeks. Vehicle-dosed chow-fed C57BL/6J mice (SC, QD, n=10) served as lean healthy controls. Tumor histopathological classification and evaluation was performed by a clinical histopathologist. Within-subject (pre-to-post) change in histopathological NAS and Fibrosis Stage was performed, Other endpoints included terminal blood biochemistry and quantitative histomorphometry.

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	15	**Vehicle**	SC	QD	-
3	DIO-MASH-HCC	Male	17	**Tirzepatide**	SC	QD	**10 nmol/kg**

Figure 1. Study outline. SC: Subcutaneous, QD; Once daily; GAN; Gubra Amylin NASH.

2 Metabolic and biochemical parameters

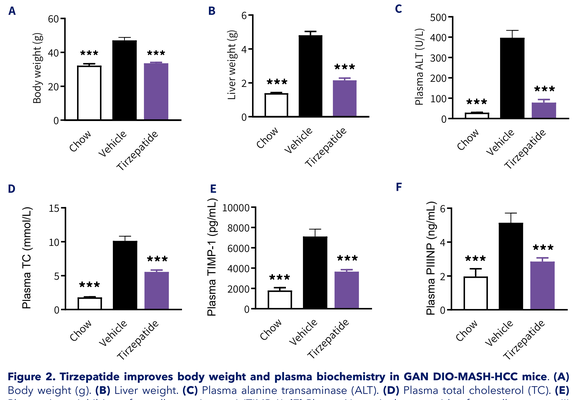


Figure 2. Tirzepatide improves body weight and plasma biochemistry in GAN DIO-MASH-HCC mice. **(A)** Body weight (g). **(B)** Liver weight. **(C)** Plasma alanine transaminase (ALT). **(D)** Plasma total cholesterol (TC). **(E)** Plasma tissue inhibitor of metalloproteinases 1 (TIMP-1). **(F)** Plasma N-terminal propeptide of procollagen type III (PIIINP). ***p<0.001 compared to DIO-MASH-HCC vehicle group (Dunnett's test one-factor linear model).

3 NAFLD Activity Score and Fibrosis Stage

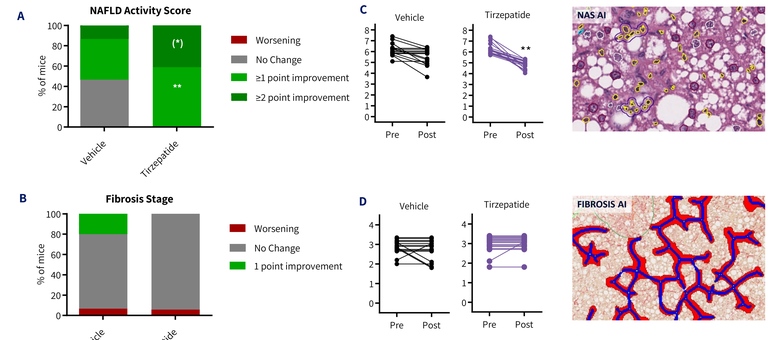


Figure 2. Tirzepatide improves NAFLD Activity Score in GAN DIO-NASH-HCC mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis stage. **(C)** Comparison of individual pre-post NAS. **(D)** Comparison of individual pre-post Fibrosis Stage. (*) p < 0.1, *p<0.05 with one-point improvement, **p<0.01 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, fibrosis & fibrogenesis

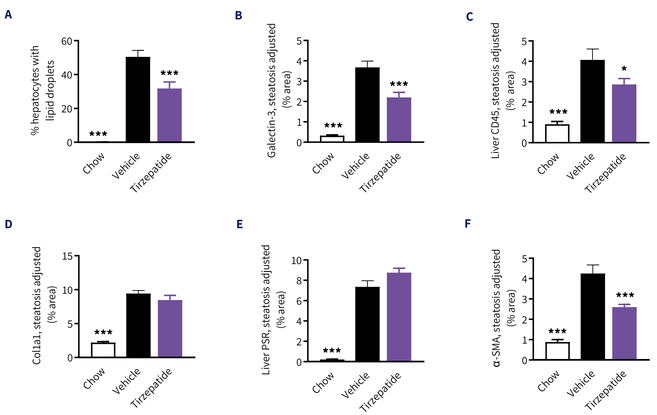


Figure 3. Tirzepatide improves quantitative histological markers of steatosis, inflammation and fibrogenesis in GAN DIO-MASH-HCC mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panel A) and conventional IHC image analysis (panels B-F, steatosis-adjusted fractional (%) area), respectively. **(A)** % hepatocytes with lipid droplets. **(B)** %-area of galectin-3. **(C)** %-area of CD45. **D)** % area of collagen-1a1. **(E)** % area of PSR. **(G)** % area of alpha-smooth muscle actin (α-SMA). Mean ± SEM. *p<0.05, ***p<0.001 compared to DIO-NASH-HCC vehicle group (Dunnett's test one-factor linear model).

5 Hepatocellular carcinoma burden

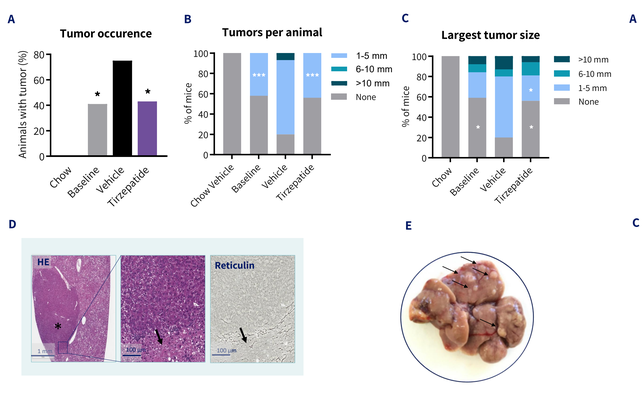


Figure 4. Tirzepatide prevents tumor progression in GAN DIO-MASH-HCC mice. **(A)** Macroscopic (surface) tumor occurence **(B)** Tumors numbers and size distribution. **(C)** Largest tumor size. **(D)** Representative images of HE and reticulin stained tumor sections. High resolution image demonstrating increased hepatocyte nuclear/cytoplasmic ratio (condensed cytoplasm with normal or enlarged nuclei) and absent reticulin trabecular framework. Asterisk marks large tumor and arrows indicates 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<0.001 compared to DIO-MASH-HCC vehicle group (Dunnett's test one-factor linear model).

6 Histological marker of proliferation

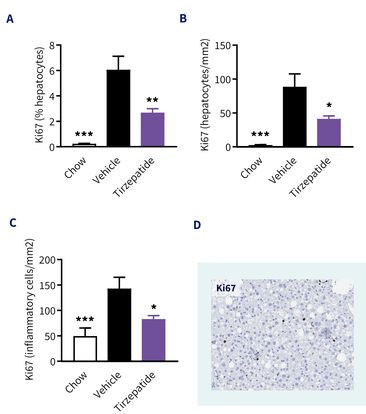


Figure 5. Tirzepatide improves quantitative histological marker of cell proliferation in GAN DIO-MASH-HCC mice. (A-C) Ki67 staining of hepatocytes (relative number, %; area, mm2) and infammatory cells (area, mm2) in non-tumorous (parenchyme) tissue. **(D)** Representative Ki67 photomicrograph (scale bar, 100 µm). **p<0.01, ***p<0.001 vs. DIO-MASH-HCC vehicle group (Dunnett's test one-factor linear model).

Conclusion

Tirzepatide exerts hepatoprotective effects in GAN DIO-MASH-HCC mice by:

+ Robustly reducing body weight.
+ Improving hepatomegaly, plasma ALT, and liver TC.
+ Promoting a ≥2-point significant reduction in NAFLD Activity Score.
+ Improving quantitative histological markers of steatosis and inflammation.
+ Reducing HCC burden.
+ While not improving fibrosis stage, tirzepatide reduced circulating markers of fibrosis (TIMP-1, PIIINP) and histological marker of fibrogenesis (α-SMA).
+ The GAN DIO-MASH-HCC mouse is highly applicable for profiling novel drug therapies targeting MASH with advanced fibrosis and HCC

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