Characterization of disease progression and pharmacological intervention in the GAN diet-induced obese mouse model of NASH with advanced fibrosis and hepatocellular carcinoma

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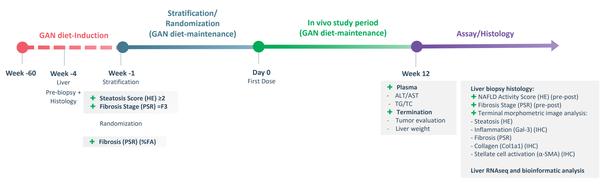
Background & Aim

Pharmacological intervention targeting metabolic-associated NASH and hepatic fibrosis might also affect hepatocellular carcinoma (HCC). The GAN (Gubra-Amylin NASH) diet-induced obese (DIO) mouse model presents with key parameters of metabolic-associated NASH including moderate hepatic fibrosis. The present study aimed to characterize disease progression in the extended GAN DIO-NASH mouse model and evaluate treatment response for the late-stage clinical drug candidate elafibranor (PPAR-α/δ agonist).

Study outline



No. #	Animal model	Group name	Number of animals	Dose [mg/kg]	Dosing Frequency	Route of administration
1	LEAN-CHOW	Chow	10	-	QD	PO
2	DIO-NASH-HCC	Vehicle	16	-	QD	PO
3	DIO-NASH-HCC	Elafibranor	14	30	QD	PO

Disease progression – Histopathology and tumor assessment

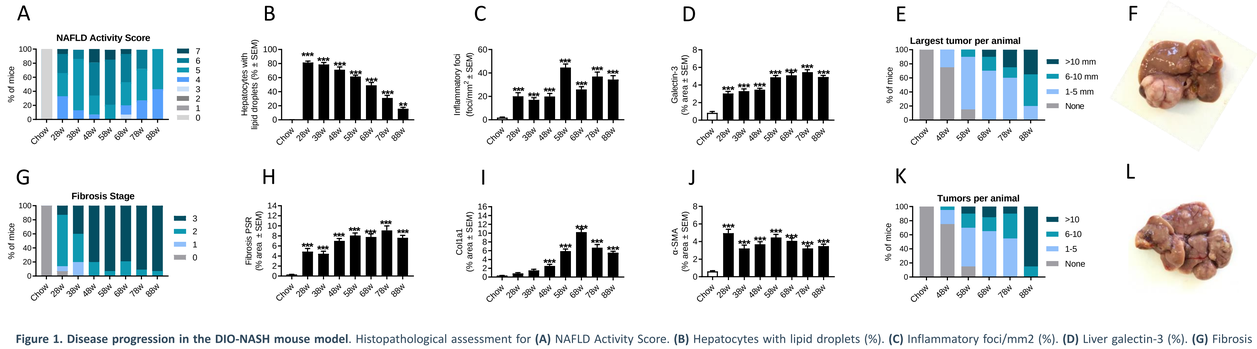


Figure 1. Disease progression in the DIO-NASH mouse model. Histopathological assessment for **(A)** NAFLD Activity Score. **(B)** Hepatocytes with lipid droplets (%). **(C)** Inflammatory foci/mm2 (%). **(D)** Liver galectin-3 (%). **(G)** Fibrosis stage. **(H)** Liver fibrosis PSR (%). **(I)** Liver Collagen 1a1 (%). **(J)** Liver α-SMA (%). Macroscopic tumor assessment for **(E)** Largest tumor for each animal. **(F)** Representative tumor images of DIO-NASH 88w. **(K)** Number of tumors per animal. **(L)** Representative tumor images of DIO-NASH 88w. **p<0.01, ***p<0.001 compared to Chow (Dunnett's test one-factor linear model).

Histopathological NAFLD Activity Score and Fibrosis Stage

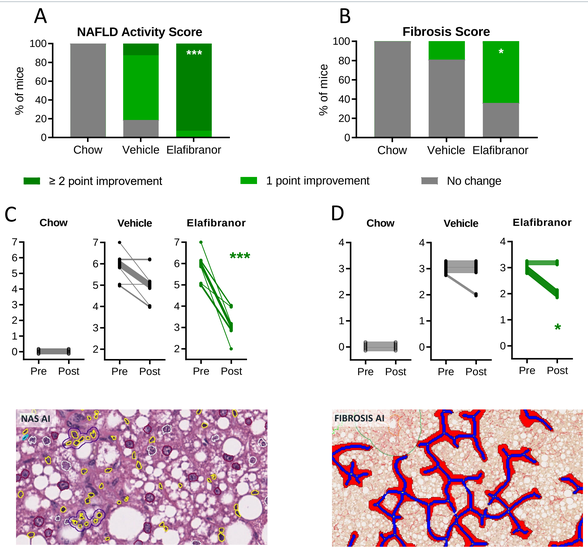


Figure 2. Elafibranor improves liver histopathological scores in DIO-NASH-HCC mice.
Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis stage. **(C)** Comparison of individual pre-post NAS. **(D)** Comparison of individual pre-post Fibrosis stage. *p<0.05, ***p<0.001 to corresponding DIO-NASH-HCC vehicle group (One-sided Fisher's exact test with Bonferroni correction). Bottom panels: Representative HE and PSR photomicrographs used for GHOST evaluation.

Histomorphometric steatosis, inflammation, fibrosis and tumor assessment

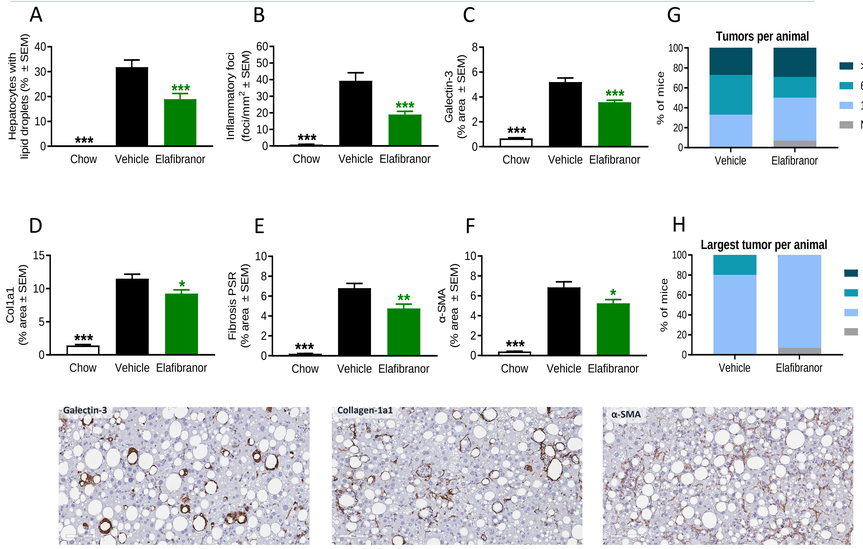


Figure 3. Elafibranor improves quantitative liver histomorphometric markers in DIO-NASH-HCC mice.
Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). **(A)** % hepatocytes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

Hepatic transcriptomic profile for HCC

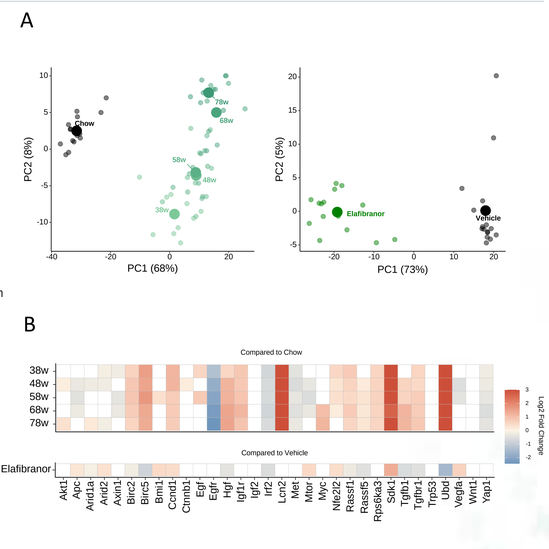


Figure 4. Elafibranor suppress hepatocellular tumor-associated genes in DIO-NASH-HCC mice.
(A) Principal component analysis (PCA) of samples based on top 500 most variable gene expression levels. **(B)** Regulation of hepatocellular tumor-associated candidate genes. GAN DIO-NASH mice for 38w, 48w, 58w, 68w and 78w compared to Chow, and Elafibranor compared to Vehicle in DIO-NASH-HCC mice. Blue and red colour gradients indicate the log2FC of significantly (p<0.05) down- and up-regulated gene expression, respectively. White boxes indicate genes not regulated (p>0.05).

CONCLUSION

- DIO-NASH mice demonstrated advanced NAFLD Activity Score (≥4) after ≥28 weeks on GAN diet.
- DIO-NASH mice progressed to bridging fibrosis (stage F3) after ≥48 weeks on GAN diet.
- DIO-NASH mice developed tumors including HCC after ≥58 weeks on GAN diet.
- DIO-NASH mice demonstrated HCC-associated transcriptomic signature.
- Elafibranor significantly improved NAFLD Activity Score and Fibrosis Stage in DIO-NASH-HCC mice.
- Elafibranor reduced histological markers of steatosis, inflammation and fibrosis in DIO-NASH-HCC mice.
- Elafibranor improved HCC-associated transcriptomic signatures in DIO-NASH-HCC mice.
- The GAN DIO-NASH-HCC mouse model is suitable for profiling novel drug therapies for advanced fibrose and HCC.