MORPHOMETRIC DIGITAL PATHOLOGY ANALYSIS REVEALS DIFFERENTIAL EFFECTS OF LANIFIBRANOR AND SEMAGLUTIDE IN THE BIOPSY-CONFIRMED GAN-DIO-NASH MOUSE MODEL

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BACKGROUND AND OBJECTIVES

Non-alcoholic steatohepatitis (NASH) predisposes to development of advanced fibrosis/cirrhosis. Many clinical trials are ongoing to obtain either significant resolution of NASH without worsening of fibrosis or improvement of fibrosis without worsening of NASH. Semaglutide (glucagon-like-receptor (GLP)-1 agonist) and lanifibranor (pan-peroxisome proliferator activated receptor agonist) are currently in late-stage clinical testing. The present study aimed at investigating the effects of these two monotherapies in the Gubra's amylin NASH (GAN) diet-induced obese (DIO) and biopsy-confirmed mouse model using morphometric digital pathology.

MATERIALS AND METHODS

GAN-DIO-NASH mice were treated with either vehicle, lanifibranor or semaglutide, and lean chow-fed animals served as control group. The pre- and post-treatment liver biopsies were stained with picrosirius red (PSR) and hematoxylin and eosin (H&E) and scanned at the magnification of X20. Histopathological NAFLD Activity Score (NAS) and fibrosis stage were evaluated by Gubra Histopathological Objective Scoring Technique (GHOST) AI-deep learning-based image analysis. In parallel, MorphoQuant, a fully automated and deterministic artificial intelligence assessed steatosis, fibrosis and collagen fiber dimensions (length and width) from PSR-stained sections. Effects of treatments were compared.

CONCLUSION

The GAN-DIO-NASH model is highly applicable for profiling novel drug therapies targeting NASH with advanced fibrosis. Notably, morphometric AI-digital pathology showed superior anti-steatotic action for lanifibranor, compared to semaglutide. In addition, the anti-fibrotic effect of lanifibranor, but not semaglutide, was demonstrated, in alignment with the Phase 2 clinical trial data. Importantly, the evaluation of collagen fiber dimensions allows to provide a better understanding of drug effect on fibrosis regression.

.01 STUDY DESIGN AND OUTLINE

Name	Animal Model	Treatment	Subject Number
Chow Vehicle	Chow	Vehicle	9
Vehicle	GAN-DIO-NASH	Vehicle	15
Lanifibranor	GAN-DIO-NASH	Lanifibranor 30 mg/kg	15
Semaglutide	GAN-DIO-NASH	Semaglutide 30 nmol/kg	16

*: p ≤ 0.05, ** : p ≤ 0.01, *** : p ≤ 0.001, ****: p ≤ 0.0001
#: p ≤ 0.05, ## : p ≤ 0.01, ### : p ≤ 0.001, ####: p ≤ 0.0001

.02 SIGNIFICANT INDUCTION OF STEATOSIS AND FIBROSIS IN GAN-DIO-NASH MOUSE MODEL AT BASELINE

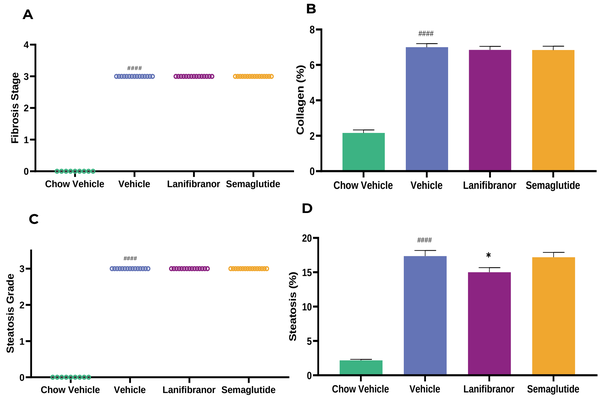

Figure 1. Fibrosis and steatosis in pre-treatment liver biopsies from GAN-DIO-NASH mice after 50 weeks of diet-induction. A. Fibrosis score by GHOST. **B.** Digital quantification of collagen by MorphoQuant. **C.** Steatosis score by GHOST. **D.** Steatosis quantification by MorphoQuant. # = t-test between chow vehicle and vehicle, * = Fisher's LSD test between vehicle and treatments

.03 SIGNIFICANT STEATOSIS REDUCTION IN LANIFIBRANOR- AND SEMAGLUTIDE-TREATED ANIMALS

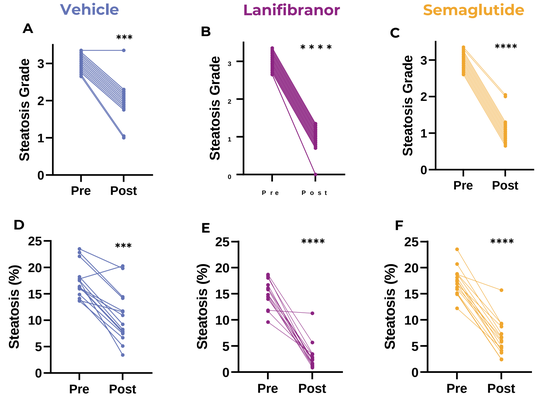

Figure 2. Assessment of steatosis response after 14-weeks treatment with Vehicle, Lanifibranor or Semaglutide in GAN-DIO-NASH mice using a Wilcoxon's test A-B-C. Steatosis score by GHOST for Vehicle-, Lanifibranor- and Semaglutide-treated animals, respectively . **D-E-F.** Steatosis quantification by MorphoQuant for Vehicle-, Lanifibranor- and Semaglutide-treated animals, respectively .

.04 AUTOMATED MORPHOMETRIC ANALYSIS OF STEATOSIS-RELATED ENDPOINTS

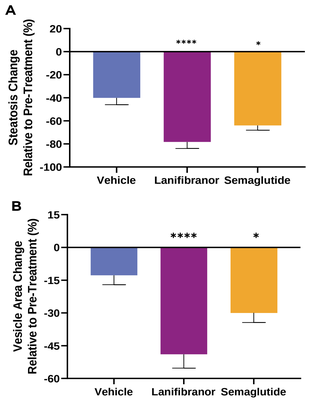

Figure 3. Automated morphometric analysis of steatosis from PSR-stained sections. A. Steatosis. **B.** Vesicle area. * = multiple comparison test betwen vehicle and treatments

.04 SIGNIFICANT FIBROSIS REGRESSION IN LANIFIBRANOR-TREATED ANIMALS

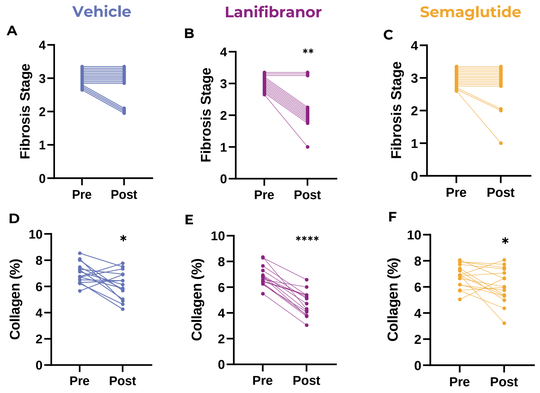

Figure 4. Assessment of fibrosis response after 14-weeks treatment with Vehicle, Lanifibranor or Semaglutide in GAN-DIO-NASH mice using a Wilcoxon's test A-B-C. Fibrosis score by GHOST for Vehicle-, Lanifibranor- and Semaglutide-treated animals, respectively . **D-E-F.** Collagen quantification by MorphoQuant for Vehicle-, Lanifibranor- and Semaglutide-treated animals, respectively .

.05 AUTOMATED MORPHOMETRIC ANALYSIS OF FIBROSIS-RELATED ENDPOINTS

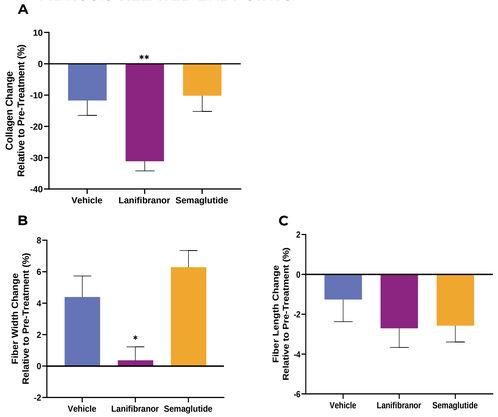

Figure 5. Automated morphometric analysis of fibrosis from PSR-stained sections. A. Collagen. **B.** Collagen width. **C.** Collagen length..

.06 MORPHOQUANT ANALYSIS OF COLLAGEN WIDTH

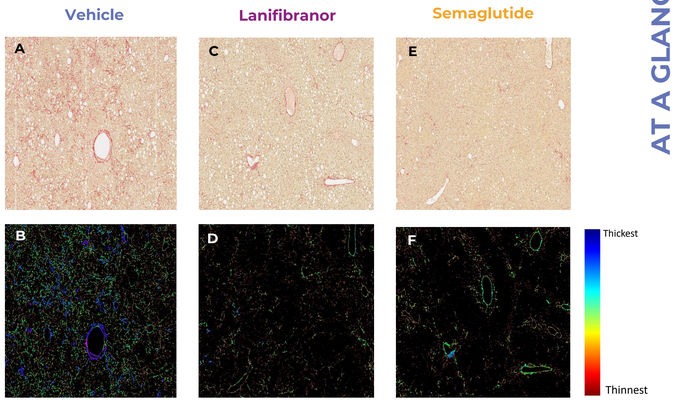

Figure 6. Rainbow color mapped images. A-B. Original image and collagen width recognition for Vehicle-treated animals. **C-D.** Original image and collagen width recognition for Lanifibranor-treated animals. **E-F.** Original image and collagen width recognition for Semaglutide-treated animals.

AT A GLANCE

- Lanifibranor and Semaglutide were investigated in GAN-DIO-NASH mouse model
- Liver biopsies were assessed for fibrosis and steatosis using GHOST and MorphoQuant (morphometric digital analysis)
- Both Lanifibranor and Semaglutide showed significant decrease of steatosis
- Only Lanifibranor reduced significantly fibrosis, preventing the thickening of collagen fibers
- GAN-DIO-NASH mouse is a highly applicable model for the investigation of NASH treatments with advanced fibrosis
- Evaluation of collagen fiber dimensions allows a better understanding of drug effect