MET409, a potent, non-bile acid sustained FXR agonist, improves NAFLD Activity Score and exerts anti-fibrotic action in a diet-induced obese mouse model of biopsy-confirmed NASH with fibrosis

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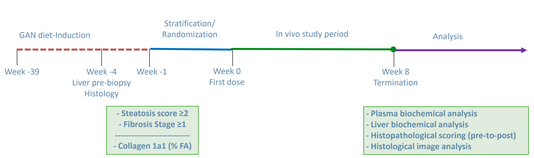
INTRODUCTION AND AIM

The farnesoid X receptor (FXR) is a ligand activated transcription factor highly expressed in the liver and intestinal tract. Sustained FXR activation has shown efficacy in clinical trials for non-alcoholic steatohepatitis (NASH); notably in conjunction with anti-fibrotic action. The aim of this study was to explore the effect of MET409, a potent non-bile acid sustained FXR agonist, on metabolic, biochemical and histopathological endpoints in a diet-induced obese (DIO) mouse model of biopsy-confirmed NASH with fibrosis.

METHODS

Male C57BL/6J mice were fed Gubra Amylin NASH (GAN)[1] diet high in fat, fructose and cholesterol for 35 weeks prior to liver pre-biopsy collection. Only animals with biopsy-confirmed steatosis (score ≥2) and fibrosis (stage ≥F1) were included. Animals were stratified and randomized into treatment groups based on liver collagen 1a1 (% fractional area). DIO-NASH mice received vehicle (PO, QD), MET409 (3 mg/kg, PO, QD) and MET409 (10 mg/kg, PO, QD) for 8 weeks. Pre-post liver biopsy histopathology was performed for within-subject evaluation of changes in composite NAFLD Activity Score (NAS) and Fibrosis Stage. Also, terminal quantitative liver histology, blood and liver biochemistry was assessed.

STUDY DESIGN



Group #	Animal	Gender	Strain	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing volume	Dosing concentration
1	DIO-NASH	Male	C57BL/6JRj	14	**Vehicle**	PO	QD	5 ml/kg	-
2	DIO-NASH	Male	C57BL/6JRj	**14**	**MET409**	PO	QD	5 ml/kg	**3 mg/kg**
3	DIO-NASH	Male	C57BL/6JRj	**14**	**MET409**	PO	QD	5 ml/kg	**10 mg/kg**

RESULTS

Baseline characteristics in GAN DIO-NASH mice

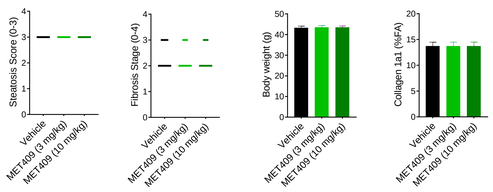


Figure 1 | Liver pre-biopsy stratification and randomization into study groups after 39 weeks on GAN diet. Inclusion criteria for GAN DIO-NASH mice were based on steatosis score (≥2), fibrosis stage (≥1) and randomization based on body weight and collagen 1a1 (% fractional area) by histomorphometric image analysis.

MET409 treatment improves metabolic and biochemical parameters in GAN DIO-NASH mice

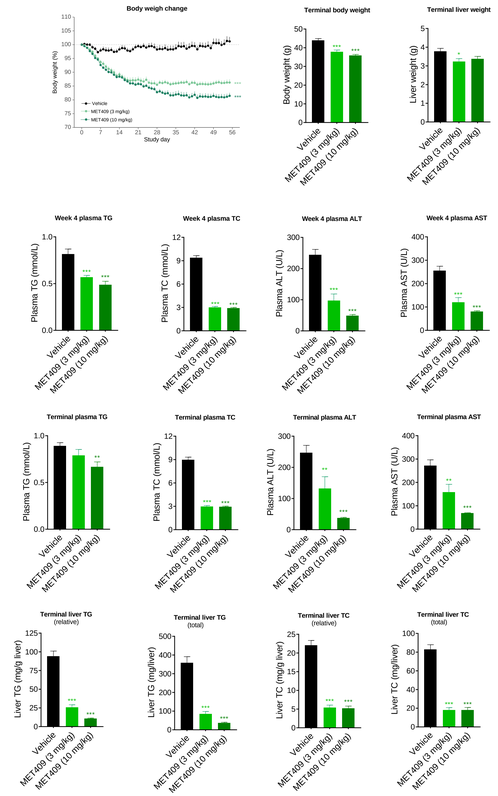


Figure 2 | Body weight change, terminal body weight, terminal liver weight and biochemical metabolic parameters. ALT: alanine transaminase, AST: aspartate transaminase, TG: total triglycerides, TC: total cholesterol. *p<0.05, **p<0.01, ***p<0.001 vs. Vehicle.

MET409 treatment reverses hepatosteatosis in GAN DIO-NASH mice

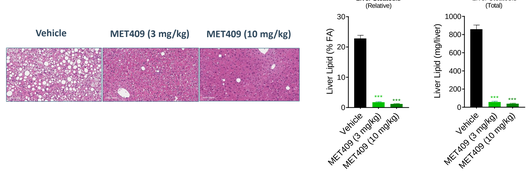


Figure 3 | Representative histological images, % fractional area (FA) and total levels of terminal liver steatosis (lipid) determined by histomorphometry. ***p<0.001 vs. Vehicle.

MET409 treatment improves NAFLD Activity Score in GAN DIO-NASH mice

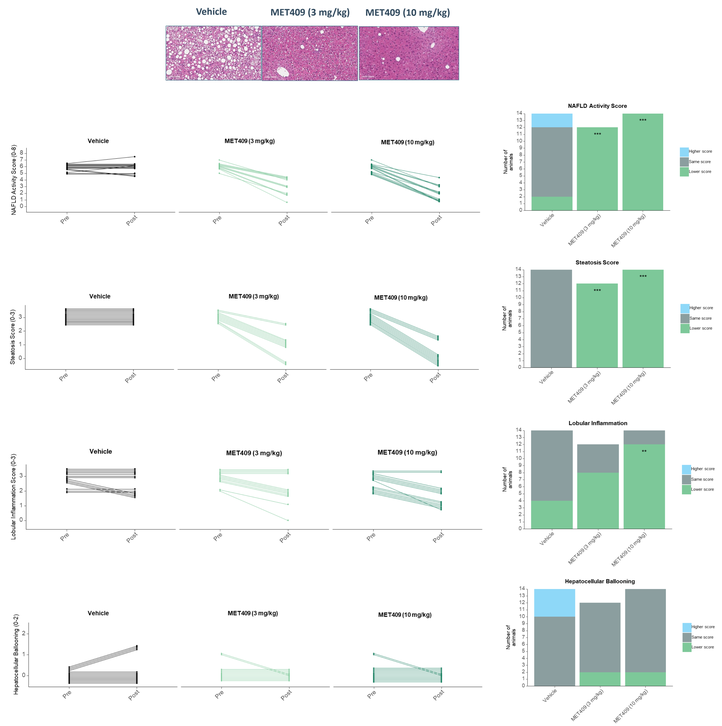


Figure 3 Histopathological scoring (pre-to-post) for liver biopsies for all animals separated by groups. For each intervention group, significance of number of animals with a lower score versus vehicle was assessed using Fisher's exact test followed by adjustment for multiple correction using the Bonferroni method. **p<0.01, ***p<0.001 vs. Vehicle group.

MET409 treatment improves Fibrosis Stage in GAN DIO-NASH mice

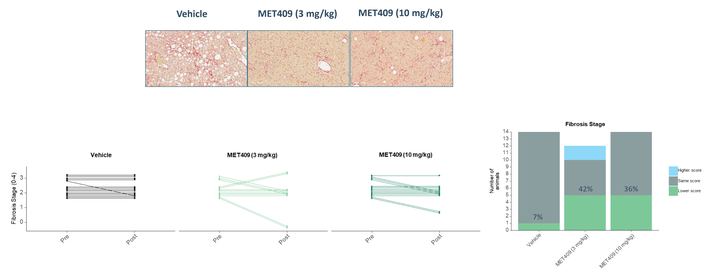


Figure 4 Histopathological scoring (pre-to-post) for liver biopsies for all animals separated by groups. For each intervention group, significance of number of animals with a lower score versus vehicle was assessed using Fisher's exact test followed by adjustment for multiple correction using the Bonferroni method.

MET409 treatment reverses hepatic inflammation and reduces hepatic fibrosis in GAN DIO-NASH mice

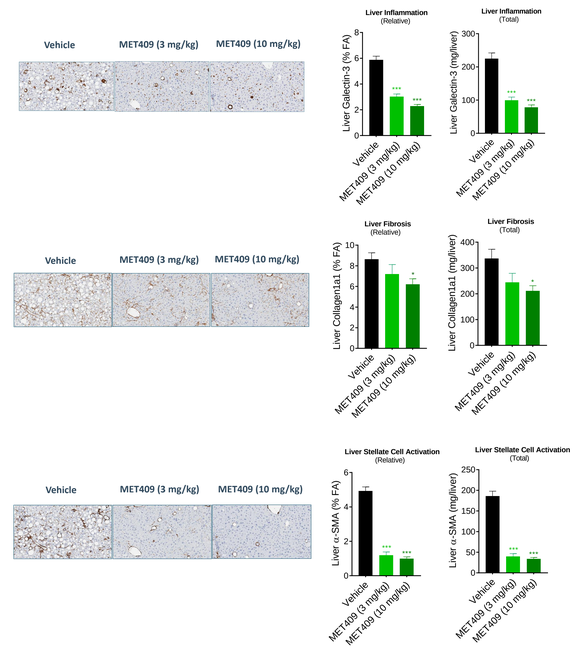


Figure 5| Representative images and % fractional area (FA) for terminal liver inflammation (Galectin-3), fibrosis (Collagen 1a1) and stellate cell activation (α-SMA) determined by histomorphometry. *p<0.05, ***p<0.001 vs. Vehicle group.

CONCLUSION

- The GAN DIO-NASH mouse model exhibits metabolic disease and biopsy-confirmed hallmarks of NASH with fibrosis.
- Treatment with the non-bile acid sustained FXR agonist MET409 for 8 weeks:
  - Improved the metabolic and biochemical profile
  - Reversed steatohepatitis by histomorphometry
  - Improved composite NAFLD Activity Score (pre-to-post)
  - Improved Fibrosis Stage (pre-to-post)
  - Reduced fibrosis and hepatic stellate cell activation by histomorphometry
- FXR agonists with similar profiles to MET409 are promising drug candidates for treatment of liver pathology by improving fibrosing NASH and preventing fibrogenesis.

[1]Boland ML et al. World J Gastroenterol. 2019:25(33). "Towards a standard diet-induced and biopsy-confirmed mouse model of non-alcoholic steatohepatitis: Impact of dietary fat source".