Additive hepatoprotective effects of semaglutide and resmetirom combination therapy in the GAN diet-induced obese and biopsy-confirmed mouse model of MASH

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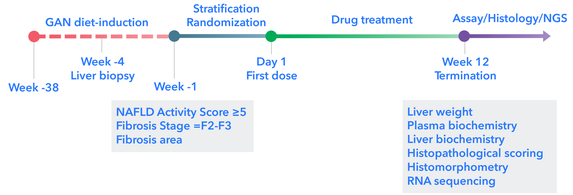
Background & Aim

Resmetirom (THR-β receptor agonist) has recently been approved for the treatment of metabolic dysfunction-associated steatohepatitis (MASH) and semaglutide (GLP-1R agonist) is in late-stage clinical development for treatment of MASH. The present study aimed to characterize metabolic, biochemical, histological and transcriptomic outcomes of resmetirom and semaglutide combination treatment in the translational GAN diet-induced obese (DIO) and biopsy-confirmed mouse model of MASH with liver fibrosis.

1 Study outline



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing frequency	Dosing concentration
1	LEAN-CHOW	Male	10	**Vehicle**	SC	QD	NA
2	DIO-MASH	Male	18	**Vehicle**	SC	QD	NA
3	DIO-MASH	Male	18	**Semaglutide**	SC	QD	30 nmol/kg
4	DIO-MASH	Male	18	**Resmetirom**	PO	QD	3 mg/kg
5	DIO-MASH	Male	17	**Semaglutide + Resmetirom**	SC + PO	QD + QD	30 nmol/kg + 3 mg/kg

Figure 1. Study outline. PO; per oral, SC; subcutaneous, QD; once daily, NA; not applicable, GAN; Gubra Amylin NASH, NGS; next generation sequencing.

2 Metabolic and biochemical parameters

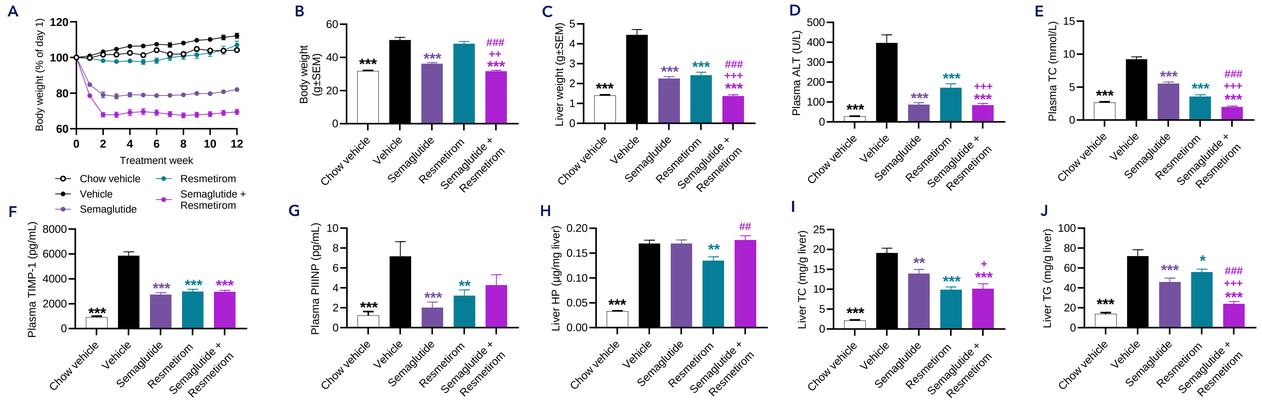
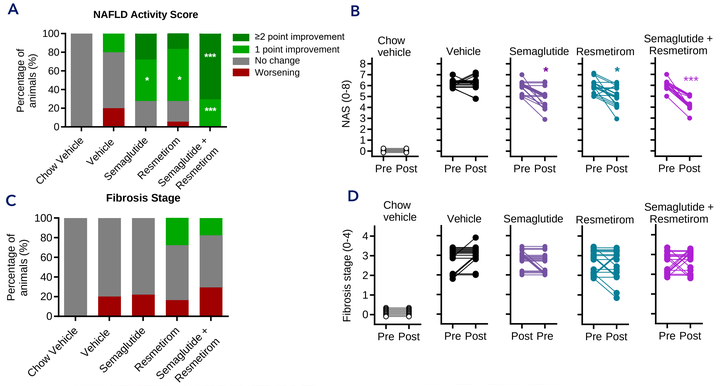


Figure 2. Semaglutide + Resmetirom combo treatment reduced weight loss, liver weight and biochemical parameters superior to mono treatments. (A) Relative body weight during study period. **(B)** Terminal body weight. **(C)** Terminal liver weight. **(D)** Terminal plasma alanine aminotransferase (ALT). **(E)** Terminal plasma total cholesterol. **(F).** Plasma tissue inhibitor of metalloproteinases (TIMP-1), **(G)** Plasma Type III Procollagen Peptide (PIIINP) **(H)** Liver hydroxyproline (HP). **(I)** Liver triglycerides (TG). **(J)** Terminal liver total cholesterol (TC). .*p<0.05, **p<0.01, ***p<0.001 compared to Vehicle. #/+p<0.05, ##/++p<0.01, ###/+++p<0.001 compared to resmetirom (#) or semaglutide (+) (Dunnett's test one-factor linear model).

3 NAFLD Activity Score and Fibrosis Stage



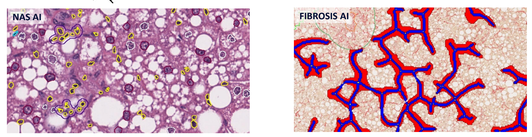
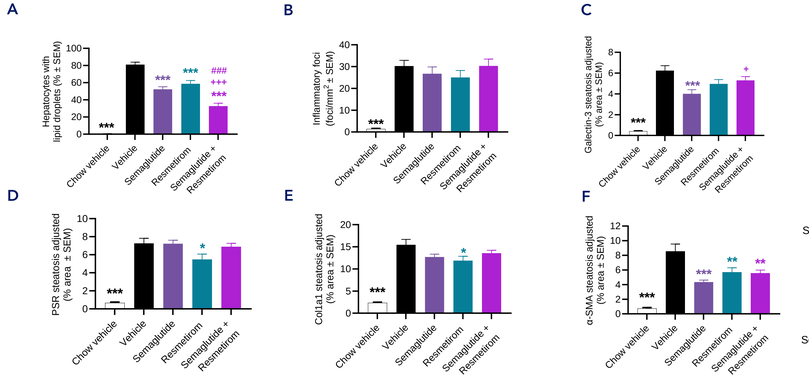


Figure 3. Semaglutide + Resmetirom combo treatment improved NAFLD Activity Score superior to mono treatments . Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Individual pre-post NAS. **(C)** Fibrosis stage. **(D)** Individual pre-post fibrosis stage. *p<0.05, ***p<0.001 compared to vehicle. (One-sided Fisher's exact test).

4 Histological markers of steatosis, inflammation and fibrosis



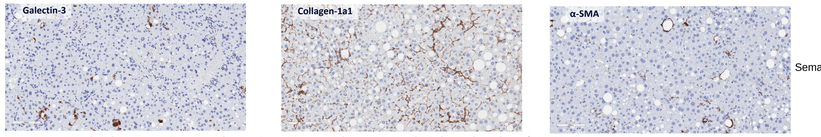
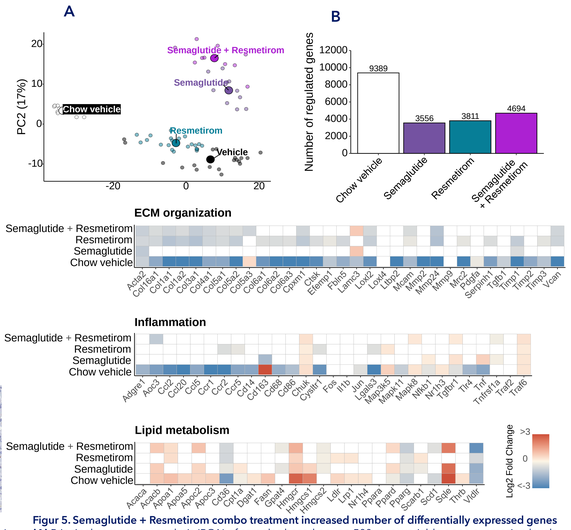


Figure 4. Semaglutide + Resmetirom combo treatment improved quantitative histological marker of steatosis superior to mono treatments. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). **(A)** % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of galectin-3 (steatosis adjusted). **(D)** % area of PSR (steatosis adjusted). **(E)** % area of collagen-1a1 (Col1a1, steatosis adjusted). **(F)** % area of alpha-smooth muscle actin (α-SMA, marker of stellate cell activation, steatosis adjusted). Mean ± SEM. *p<0.05, ***p<0.001 compared to vehicle. . #/+p<0.05, ###/+++p<0.001 compared to resmetirom (#) or semaglutide (+) (Dunnett's test one-factor linear model) . Bottom panels: Representative photomicrographs of galectin-3, α-SMA and Col1a1 (scale bar, 100 µm).

5 Hepatic transcriptomic profile



Figur 5. Semaglutide + Resmetirom combo treatment increased number of differentially expressed genes (A) Principal component analysis (PCA) of samples based on top 500 most variable gene expression levels. **(B)** Differentially expressed genes. **(C)** Regulation of hepatic extracellular matrix (ECM), inflammation and lipid metabolism candidate genes (log2-fold change compared to corresponding DIO-NASH vehicle control mice). Blue and red colour gradients indicate significantly (p<0.05) down-regulated and up-regulated gene expression, respectively. White boxes indicate genes not significantly regulated (p>0.05).

Conclusion

- Semaglutide + Resmetirom combo treatment promote superior weight loss to mono treatments.
- Semaglutide + Resmetirom combo treatment improved hepatomegaly and plasma/liver lipids superior to mono treatments.
- Semaglutide + Resmetirom combo treatment improved NAS (2-point) superior to mono treatments.
- Semaglutide + Resmetirom combo treatment reduces steatosis superior to mono treatments.
- Only resmetirom mono treatment reduces markers of fibrosis.
- Semaglutide + Resmetirom combo treatment amplified hepatic transcriptomic profile.

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