Prophylactic and therapeutic effects of the dual FXR/TGR5 agonist INT-767 in the GAN diet-induced ob/ob mouse model of advanced MASH with progressive fibrosis

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Background & Aim

The farnesoid X receptor (FXR) and G-protein-coupled bile acid receptor (TGR5, Gpbar1) have both been implicated in the pathogenesis of metabolic dysfunction-associated steatohepatitis (MASH).

The present study aimed to evaluate the efficacy of the dual FXR/TGR5 agonist INT-767 on therapeutic outcomes in an accelerated genetically obese mouse model of MASH with progressive fibrosis.

Methods

See Fig. 1 for study outline. Male leptin-deficient male B6.V-Lep^{ob}/JRj (ob/ob) mice were fed the GAN diet high in fat, fructose, and cholesterol for total of 12 or 24 weeks. GAN ob/ob-MASH mice received treatment from the first day of GAN diet feeding (prophylactic treatment) or after 12 weeks of GAN diet feeding (therapeutic intervention). GAN ob/ob-MASH mice were randomized into treatment groups according to body weight prior to study start. Animals received once daily oral dosing with INT-767 (10 mg/kg) or vehicle (n=14 per group) for 12 weeks. Terminal endpoints included plasma biomarkers, liver biochemistry, NAFLD Activity Score (NAS), fibrosis stage and quantitative liver histology.

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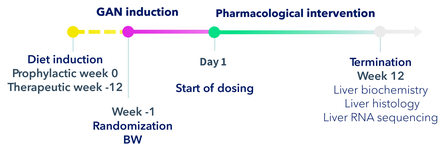
1 Study Outline



Group no.	Group	Name	Number of animals	Administration route	Dosing frequency	Dosing concentration
1	Vehicle 12w	**Vehicle (proph)**	14	PO	QD	-
2	INT-767 Prophylactic	**INT-767 (proph)**	14	PO	QD	10mg/kg
3	Vehicle 24w	**Vehicle (ther)**	14	PO	QD	-
4	INT-767 Therapeutic	**INT-767 (ther)**	14	PO	QD	10mg/kg

Figure 1. Study outline in GAN ob/ob-NASH mice

2 Metabolic and biochemical parameters

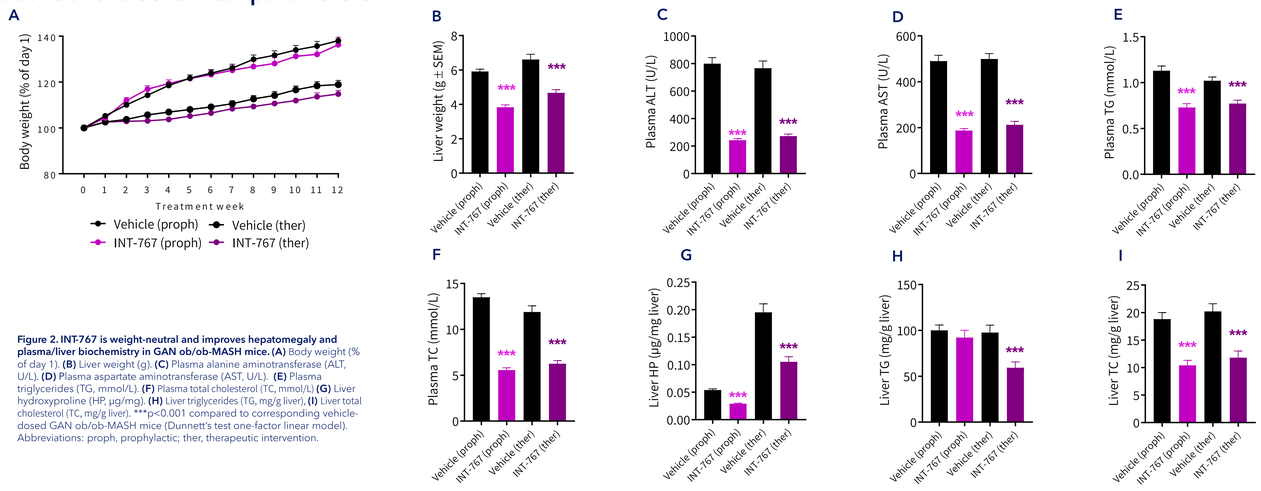


Figure 2. INT-767 is weight-neutral and improves hepatomegaly and plasma/liver biochemistry in GAN ob/ob-MASH mice. (A) Body weight (% of day 1). **(B)** Liver weight (g). **(C)** Plasma alanine aminotransferase (ALT, U/L). **(D)** Plasma aspartate aminotransferase (AST, U/L). **(E)** Plasma triglycerides (TG, mmol/L). **(F)** Plasma total cholesterol (TC, mmol/L) **(G)** Liver hydroxyproline (HP, µg/mg). **(H)** Liver triglycerides (TG, mg/g liver), **(I)** Liver total cholesterol (TC, mg/g liver). ***p<0.001 compared to corresponding vehicle-dosed GAN ob/ob-MASH mice (Dunnett's test one-factor linear model). Abbreviations: proph, prophylactic; ther, therapeutic intervention.

3 NAFLD Activity Score and Fibrosis Stage

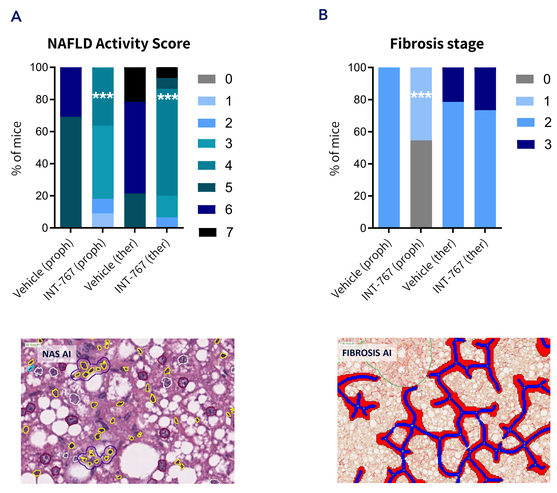


Figure 2. INT-767 intervention improves NAS and fibrosis stage in GAN ob/ob-MASH mice. Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis Stage. ***p<0.001 compared to corresponding ob/ob MASH vehicle group (One-sided Fisher's exact test with Bonferroni correction). Bottom panels: Representative HE and PSR photomicrographs used for GHOST evaluation. Abbreviations: proph, prophylactic; ther, therapeutic intervention.

4 Quantitative histological markers of steatosis, inflammation and fibrosis

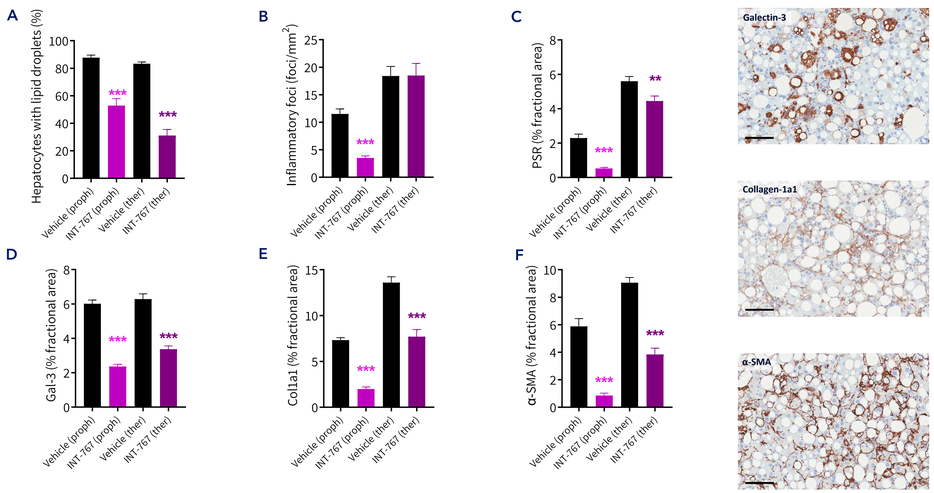


Figure 3. INT-767 improves quantitative histological markers of steatosis, inflammation and fibrosis in GAN ob/ob-MASH mice. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables. Other quantitative histological endpoints were assessed by conventional IHC image analysis **(A)** % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of PSR. **(D)** % area of galectin-3 (Gal-3). **(E)** % area of collagen-1a1 (Col1a1). **(F)** % area of alpha-smooth muscle actin (α-SMA). Mean ± SEM. **p<0.01, ***p<0.001 compared to corresponding vehicle-dosed GAN ob/ob-MASH mice (Dunnett's test one-factor linear model). Right panels: Representative photomicrographs of galectin-3, collagen 1a1 and α-SMA staining (scale bar, 100 µm). Abbreviations: proph, prophylactic; ther, therapeutic intervention.

Conclusion

INT-767 improves NASH and fibrosis in GAN ob/ob-MASH mice:

+ Prophylactic and therapeutic intervention both improves hepatomegaly and liver/plasma biochemistry
+ Prophylactic and therapeutic intervention both improves NAFLD Activity Score (NAS)
+ Prophylactic and therapeutic intervention both improves quantitative histological markers of steatosis, inflammation, and fibrosis
+ Only prophylactic treatment improves fibrosis stage

Dual FXR/TGR5 activation may have therapeutic potential in MASH. Accelerated MASH and fibrosis in GAN ob/ob-MASH mice makes this model highly instrumental in preclinical drug discovery.

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