Metabolic, biochemical and histological effects of INT-767 in the GAN diet-induced and biopsy-confirmed ob/ob mouse model of NASH

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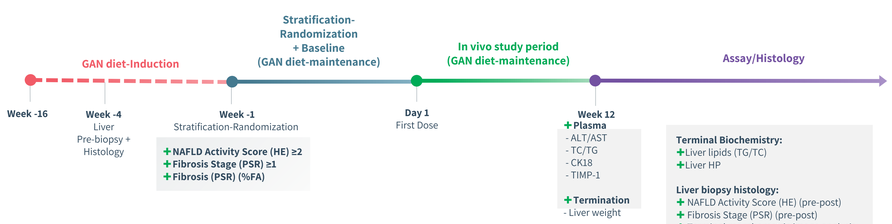
BACKGROUND & AIM

The bile acid analog INT-767 is a dual FXR/TGR5 agonist developed for the treatment of metabolic and liver diseases. Preclinical studies have previously reported the beneficial metabolic effect along with an anti-fibrotic and anti-inflammatory action, which makes this compound a potential candidate for treatmet of non-alcoholic steatohepatitis (NASH).

The present study aimed to evaluate the metabolic, biochemical, and histological effects of 12 weeks treatment with INT-767 in the biopsy-confirmed GAN diet-induced ob/ob mouse model of NASH with progressive fibrosis.

1 Study outline



Group #	Group name	Number of Animals	Animal model	Dose	Dosing frequency	Route of administration
1	Vehicle	13	ob/ob-NASH	NA	QD	PO
2	INT-767	9	ob/ob-NASH	10 mg/kg	QD	PO

Figure 1. Study outline, groups and treatments.
BW: body weight ; QD: once daily; PO: per oral; ALT: alanine aminotransferase; AST: aspartate transaminase; TC: total cholesterol; TG: triglycerides; HP: Hydroxyproline; CK18: cytokeratin-18; TIMP-1: Tissue inhibitor of metalloproteinase-1; HE: Haematoxylin Eosin; PSR: Picro Sirius Red; IHC: Immunohistochemistry .

2 Metabolic and biochemical parameters

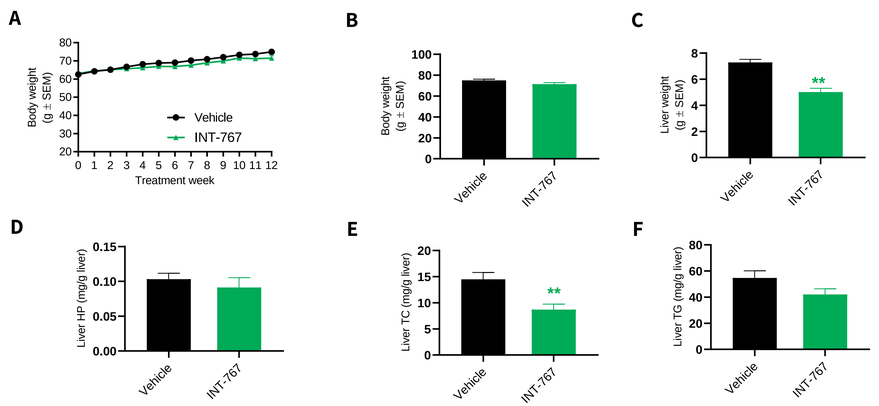


Figure 2. INT-767 improves metabolic and biochemical parameters in GAN ob/ob-NASH mice.
(A) Absolute body weight during study period. **(B)** Terminal body weight. **(C)** Terminal liver weight. **(D)** Terminal liver hydroxyproline. **(E)** Terminal liver total cholesterol. **(F)** Terminal liver triglycerides. **p<0.01, ***p<0.001 compared to corresponding vehicle control (Dunnett's test one-factor linear model).

3 Plasma biomarkers

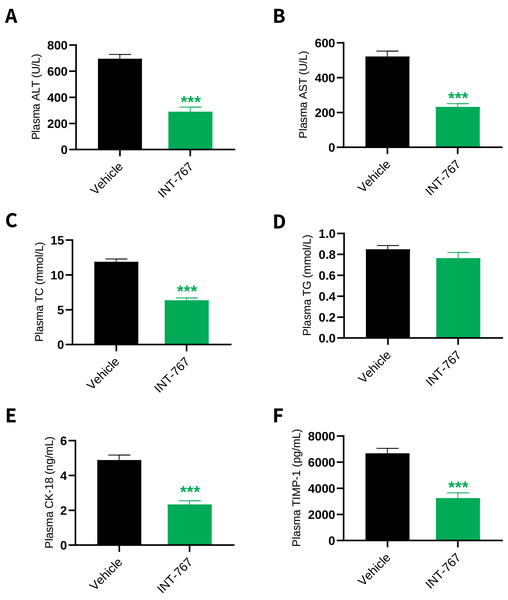


Figure 3. INT-767 improves plasma biomarkers in GAN ob/ob-NASH mice.
(A) Terminal plasma alanine aminotransferase (ALT). **(B)** Terminal plasma aspartate aminotransferase (AST). **(C)** Terminal liver total cholesterol. **(D)** Terminal plasma triglycerides. **(E)** Terminal plasma cytokeratin-18. **(F)** Terminal plasma TIMP metallopeptidase inhibitor 1. ***p<0.001 compared to corresponding vehicle control (Dunnett's test one-factor linear model).

4 Histopathological NAFLD Activity Score and Fibrosis Stage

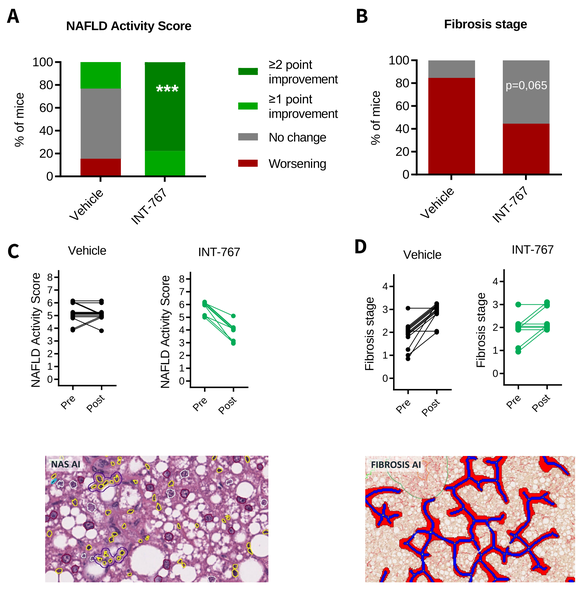


Figure 3. INT-767 improves NAFLD Activity Score in GAN ob/ob-NASH mice.
Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis stage. **(C, D)** Comparison of individual pre-post NAS and individual pre-post Fibrosis stage. ***p<0.001 to corresponding DIO-NASH vehicle group (One-sided Fisher's exact test with Bonferroni correction). Bottom panels: Representative HE and PSR photomicrographs used for GHOST evaluation.

5 Histological quantitative markers of steatosis, fibrosis and inflammation

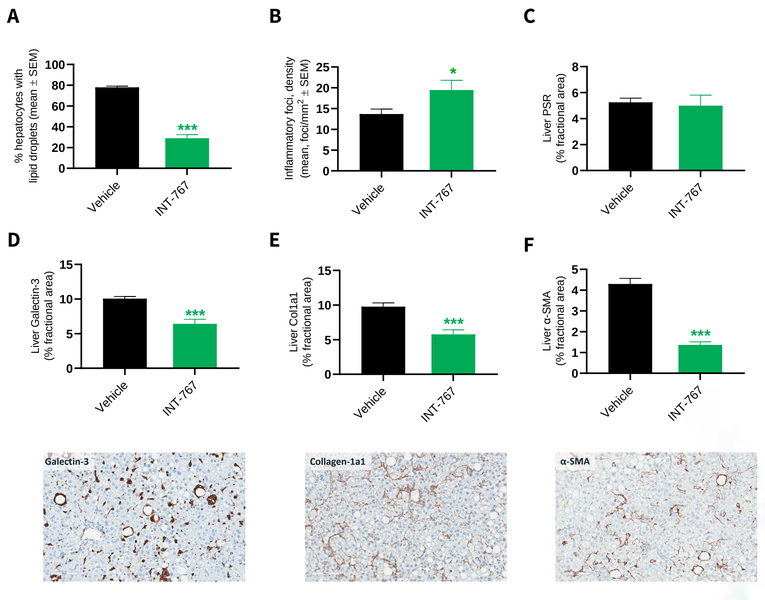


Figure 5. INT-767 decreases histological markers for steatosis, inflammation and fibrogenesisin GAN ob/ob-NASH mice.
Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). **(A)** % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of PSR. **(D)** % area of galectin-3. **(E)** % area of collagen-1a1. **(F)** % area of alpha-smooth muscle actin (α-SMA) as marker for stellate cell activation. Mean ± SEM. *p<0.05, ***p<0.001 to corresponding vehicle group (Dunnett's test one-factor linear model). Bottom panels: Representative galectin-3, collagen 1a1 and α-SMA photomicrographs for INT-767 treatment group (scale bar, 100 µm).

CONCLUSION

- INT-767, irrespectively of weight loss, improved hepatomegaly and reduced liver total cholesterol.
- INT-767 improved plasma biomarkers including ALT/AST, TC, CK18 and TIMP-1
- INT-767 promoted ≥2-point improvement in NAFLD Activity Score.
- INT-767 prevented worsening in fibrosis stage.
- INT-767 reduced histological markers for liver inflammation, collagen deposition and stellate cell activation.
- These findings demonstrate metabolic and histopathological benefits of INT-767 in the GAN diet-induced ob/ob-NASH mouse model and validate FXR/TGR5 as pharmacological targets for NASH.

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