Detailed Analysis of the Antifibrotic effects of INT-767, a dual FXR/TGR5 agonist, in an obese mouse model of diet-induced and biopsy-confirmed NASH

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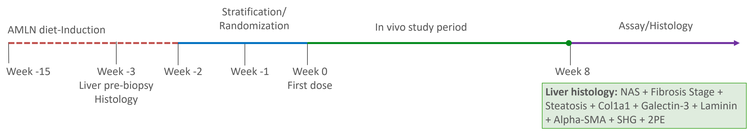
Introduction and Aim

The farnesoid X receptor (FXR) and G protein coupled receptor (GPCR) TGR5 have been implicated in the regulation of metabolism, inflammation and fibrosis in non-alcoholic steatohepatitis (NASH). A dual FXR/TGR5 agonist, INT-767, has been developed and is in early clinical development. Here we evaluated the efficacy of INT-767 on qualitative and qualitative histological parameters in an obese mouse model of diet-induced and biopsy-confirmed NASH.

Methods

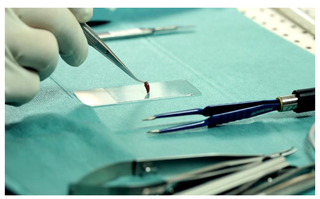
Male leptin-deficient Lep$^{ob/ob}$ mice (5 weeks of age) were fed a diet high in trans-fat, fructose and cholesterol (ob/ob-NASH). Livers were biopsied after 12 weeks on diet, and mice with steatosis (score 2-3) and fibrosis (stage 1-3) were randomized (n=10/group) to receive vehicle (PO, QD) or INT-767 (10 mg/kg, PO, QD) for 8 weeks. Primary endpoints included a blinded histological evaluation of fibrosis, total NAFLD Activity Score (NAS) and its components. Liver fibrosis was analyzed using picro-Sirius Red (SR) staining and Col1a1 immunohistochemistry. Second harmonic generation (SHG) microscopy was used to quantify collagen fiber density (total brightness of collagen intensity) and collagen structure network (skeletonization, branch nodes). Lipid deposition was estimated by H&E staining and two-photon excitation fluorescence (2PE), with droplet size by 2PE. Immunohistochemical staining for fractional area laminin (basement membrane remodeling), galectin-3 (macrophages) and alpha-SMA (activated hepatic stellate cells) was also assessed.

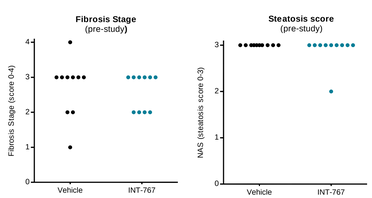
Study Design



Group #	Animal	Gender	Strain	Number of animals	Treatment	Administration form	Dosing volume	Daily Dosing concentration
1	ob/ob-NASH	Male	Lep$^{ob/ob}$	10	**Vehicle**	QD, PO	5 ml/kg	-
2	ob/ob-NASH	Male	Lep$^{ob/ob}$	10	**INT-767**	QD, PO	5 ml/kg	**10 mg/kg**

Liver pre-biopsy procedures and in vivo efficacy





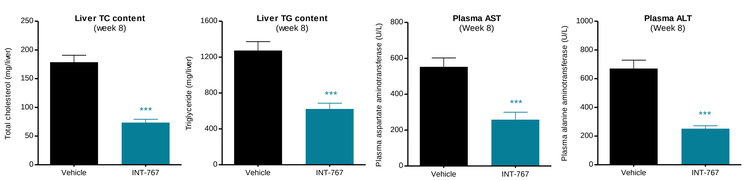


Figure 1
Animals were included based on a pre-study (week -3) histological assessment of steatosis stage and fibrosis score and subsequently randomized into treatment groups. INT-767 showed clear effect on liver total cholesterol (TC), liver triglyceride (TG), as well as plasma aspartate amino transferase (AST) and alanine amino transferase (ALT).
Data presented as mean ± SEM. ***p<0.001 (unpaired t-test).

NASH histopathology and morphometry

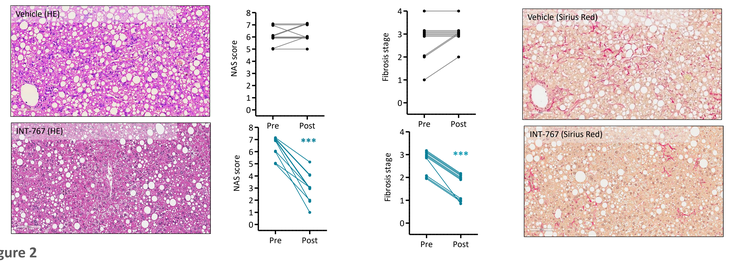


Figure 2
Histopathological scoring of the pre- and post-study biopsies for NAFLD Activity Score (NAS) and Fibrosis Stage in ob/ob-NASH mice. INT-767 treatment for 8 weeks significantly improved liver histopathology in mice with biopsy-confirmed liver pathology. ***p<0.001 (Chi square test, vs. vehicle).

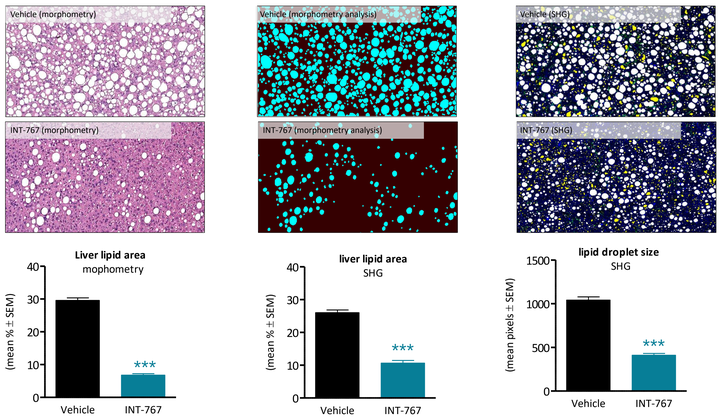


Figure 3
Morphometric analyses of liver fat area (morphometry) compared to 2-PE analysis of fat area, and mean fat droplet size. INT-767 showed a strong effect on fat assessed by both methodologies). Fat droplets (white), rejected structures (yellow), collagen (green), auto-fluorescence (blue). Data presented as mean ± SEM. ***p<0.001 (unpaired t-test).

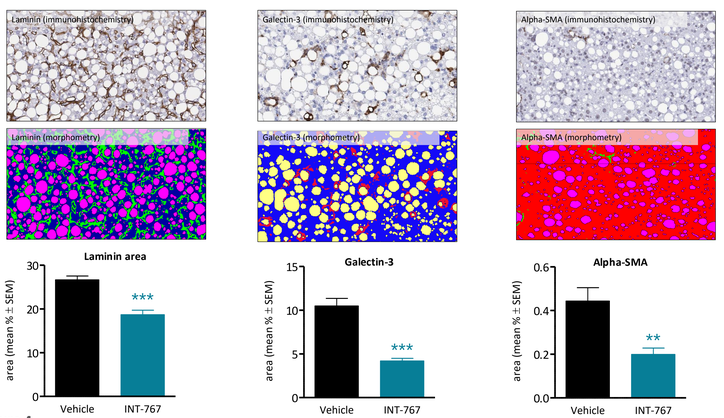


Figure 4
Morphometry analysis of laminin, galectin-3 and alpha-smooth muscle actin (alpha-SMA) content. INT-767 treatment significantly reduced all parameters.
Data presented as mean ± SEM. **p<0.01, ***p<0.001 (unpaired t-test).

Quantification of collagen deposition

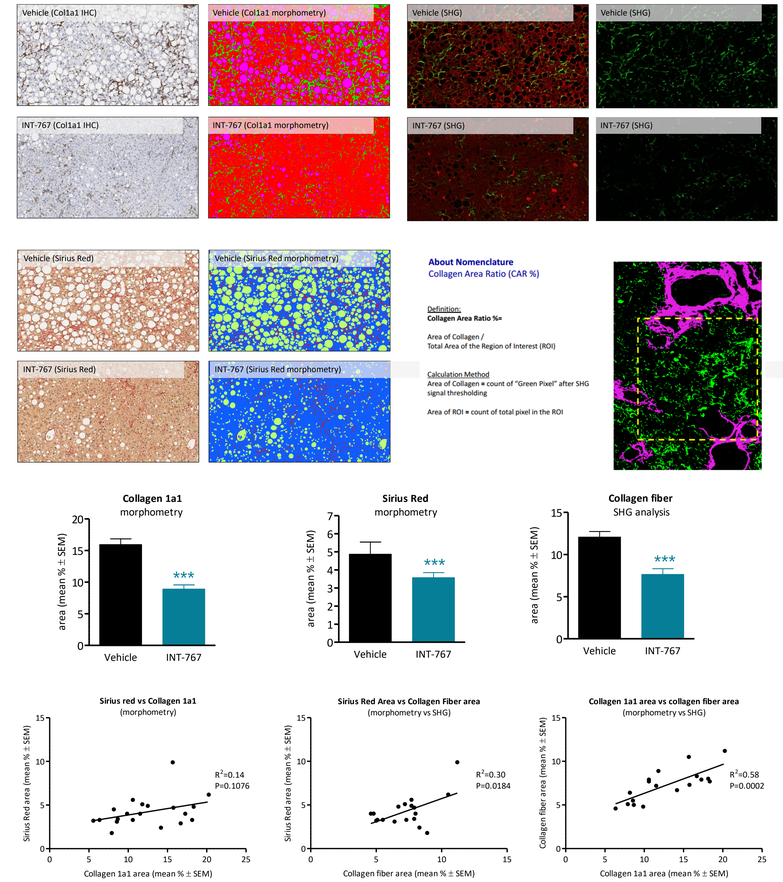


Figure 5
Comparative image analyses of collagen content. Collagen content was determined by morphometric analyses of Col1a1 immunohistochemistry and Sirius Red, and compared to SHG analysis of collagen fiber content and collagen fiber density. INT-767 reduced collagen content significantly and tended to reduced collagen fiber density (mean pixel intensity). A clear correlation waa observed across stainings and methodologies. Data are expressed as % of total parenchymal area (subtraction of fat area). ***p<0.001 (unpaired t-test).

Conclusion

- **INT-767 treatment improved liver histopathology in ob/ob-NASH mice by improving NAS and Fibrosis Stage**
- **INT-767 decreased liver lipid load and reduced mean lipid droplet size**
- **INT-767 decreased basal membrane fibrosis (laminin), inflammation (galectin-3) and activated hepatic stellate cells (alpha-SMA)**
- **Quantitative Collagen 1a1 immunohistochemistry (IHC) or label-free SHG/2PE are reasonable surrogates for Sirius Red. These newer techniques provide improved thresholding for signal detection and in the case of SHG/2PE further opportunity for morphometrics**
- **These preclinical findings provide proof-of concept for FXR/TGR5-based therapies in NASH. Furthermore these findings demonstrate the added value of using multiple quantitative imaging methodologies to complement NASH histopathological scores**