Targeting nuclear envelope improves NAFLD Activity Score and shows antifibrotic and hepatoprotective effects superior to Lanifibranor in the GAN diet-induced obese and biopsy-confirmed mouse model of MASH

Authors

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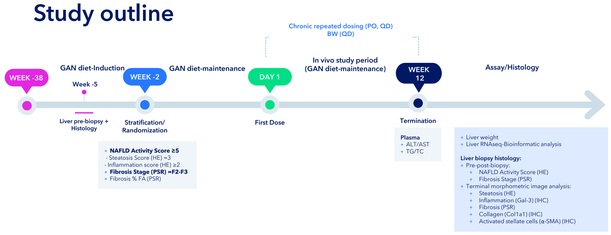
Background & Aim

NLT-101 is a first-in-class compound that functions by proximity engagement with a target in the nuclear envelope that mediates its activity. NLT-101 is in preclinical development for metabolic dysfunction-associated steatohepatitis (MASH) and fibrosis driven indications.

The present study aimed to investigate the therapeutic benefits of NLT-101 on metabolic, biochemical, histopathological and transcriptome profile in the translational GAN diet-induced obese (DIO) and biopsy-confirmed mouse model of MASH with liver fibrosis. Lanifibranor, a late-stage clinical candidate (Ph3) for the treatment of patients with MASH and liver fibrosis.

1 Study outline



Group	Animal	Gender	Number of animals	Treatment	Administration route	Dosing Frequency	Dosing Administered
1	Lean-chow	Male	10	**NA**	PO	QD	0
2	DIO-MASH	Male	16	**Vehicle**	PO	QD	0
3	DIO-MASH	Male	16	**NLT-101 Low**	PO	QD	30 mg/kg
4	DIO-MASH	Male	12	**NLT-101 High**	PO	QD	60 mg/kg
5	DIO-MASH	Male	14	**Lanifibranor**	PO	QD	30 mg/kg

Figure 1. Study outline. PO: Oral, QD; Once daily; GAN; Gubra Amylin NASH.

2 Metabolic and biochemical parameters

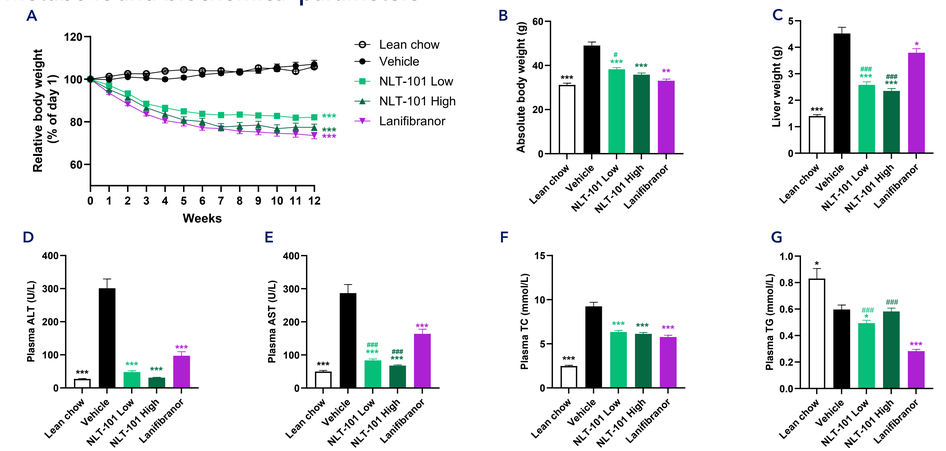


Figure 2. Metabolic and biochemical effects of NLT-101 and Lanifibranor in GAN DIO-MASH mice. (A) Relative body weight during study period. **(B)** Terminal absolute body weight **(C)** Liver weight. **(D)** Plasma alanine aminotransferase (ALT). **(E)** Plasma aspartate aminotransferase (AST) **(F)** Plasma total cholesterol (TC). **(G)** Plasma triglycerides (TG) *p<0.05, **p<0.01, ***p<0.001 compared to vehicle control, ###p<0.001 compared to Lanifibranor (Dunnett's test one-factor linear model).

3 NAFLD Activity Score and Fibrosis Stage

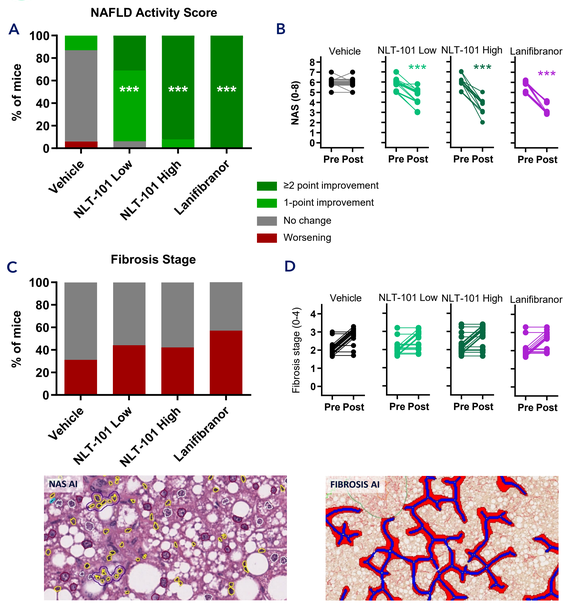


Figure 3. NTL-101 and Lanifibranor improve NAFLD Activity Score in GAN DIO-MASH mice Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Individual pre-post NAS. **(C)** Fibrosis stage. **(D)** Individual pre-post fibrosis stage. **p<0.01, ***p<0.001 compared to vehicle (One-sided Fisher's exact test).

4 Histological markers of steatosis, inflammation, fibrosis and fibrogenesis

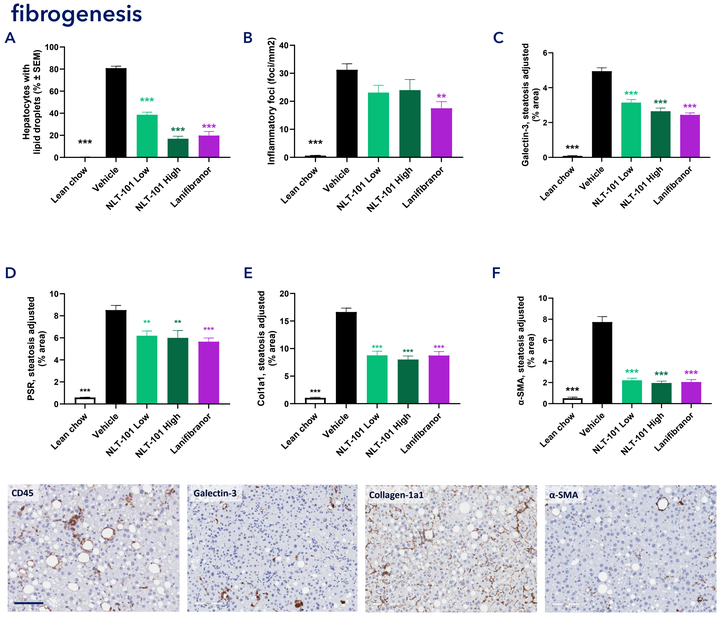


Figure 4. NLT-101 and Lanifibranor improve quantitative histological endpoints. Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables (panels A-B) and conventional IHC image analysis (panels C-F). **(A)** % hepatocytes with lipid droplets. **(B)** Inflammatory foci/mm2 **(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). *p<0.05, ***p<0.001 compared to vehicle control (Dunnett's test one-factor linear model). Bottom panels: Representative photomicrographs of CD45 Gal-3, Col1a1 and α-SMA (scale bar, 100 µm).

5 Hepatic transcriptome analysis

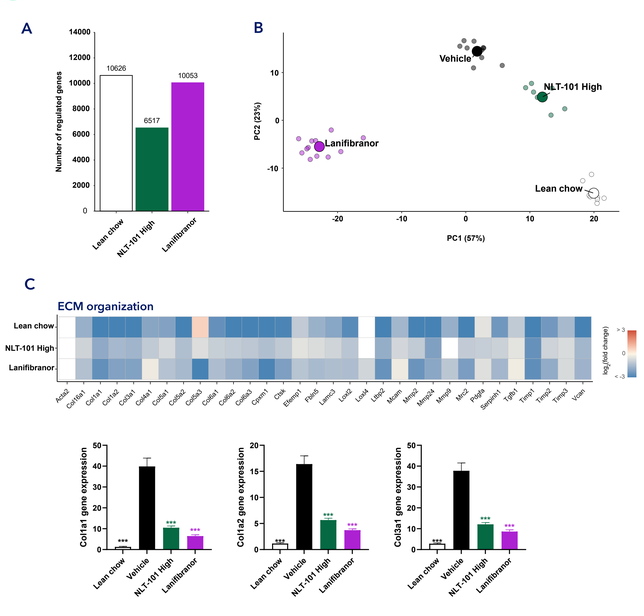


Figure 5. Hepatic transcriptome signatures following NLT-101 and Lanifibranor therapy. (A) Total number of differentially expressed genes. **(B)** Principal component analysis based on the top 500 most varying genes across the dataset **(C)** Regulation of gene expression in the extracellular matrix (ECM). Log2 fold change is indicated for significantly regulated genes ($p_{adj} < 0.05$ after correcting for multiple testing). *p<0.05, **p<0.01, ***p<0.001 . Red and blue colours indicate up- and down-regulation, respectively, compared to Vehicle.

Conclusion

- NLT-101 at both doses (30 mg/kg and 60 mg/kg) reduced body weight in GAN DIO-MASH mice, similarly to Lanifibranor.
- NLT-101 improved hepatomegaly and plasma transaminases superior to Lanifibranor.
- NLT-101 improved plasma cholesterol levels similarly to Lanifibranor.
- NLT-101 improved improved NAFLD Activity Score similarly to Lanifibranor.
- The therapeutic benefits of NLT-101 on quantitative histological hallmarks of MASH (steatosis, inflammation) were comparable to Lanifibranor.
- NLT-101 reduced quantitative histology for fibrosis and fibrogenesis similarly to Lanifibranor.
- NLT-101 impacted transcriptomic profile and significantly modulated gene expression markers for ECM organization.
- NLT-101 is a promising first-in-class nuclear targeting compound for the treatment of MASH with liver fibrosis.

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