WED-445

Combination therapy of TERN-501, a selective agonist of thyroid hormone receptor (THR) beta with TERN-101, a farnesoid X receptor (FXR) agonist improves nonalcoholic steatohepatitis (NASH) in a GAN diet-induced and biopsy-confirmed mouse model

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Background and Aims: Nonalcoholic steatohepatitis (NASH) is a serious disease of the liver that will likely require a combination therapy to achieve maximal therapeutic response. TERN-501, a potent and selective agonist of thyroid hormone receptor (THR) beta, and TERN-101, a non-steroidal agonist of farnesoid X receptor (FXR), were tested alone and in combination in a Gubra-Amylin NASH (GAN) diet-induced obese mouse model of NASH (DIO-NASH).

Method: TERN-101 (10 mg/kg, PO) and TERN-501 (0.3 [Low], 2 [Med], and 10 [High] mg/kg, PO) were administered once daily as single agents or in combination in biopsy-confirmed GAN DIO-NASH mice (n=16/group) for 12 weeks. Histological analyses were performed at baseline and end of treatment to assess steatosis, inflammation, and fibrosis on stained biopsies. Liver biopsies were also assessed by stain-free artificial intelligence (AI)-based digital pathology (HistoIndex®) using second harmonic generation and two photon emission.

Results: The NAFLD Activity Score (NAS) was improved to a greater extent by combination treatment with 19%, 25%, and 43% of mice showing ≥2-pt NAS improvement from baseline in the Low, Med, and High combination arms, respectively. Quantitative liver histomorphometry on stained biopsies showed the combination treatment had greater anti-steatotic activity, including reduced liver lipids, fewer hepatocytes containing lipid droplets, and reduced lipid droplet size. Analyses by HistoIndex indicated that the combination treatment significantly lowered fibrosis colocalized with macrosteatotic vesicles and reduced the progression of fibrosis colocalized with microsteatotic vesicles. The perisinusoidal area showed significant reduction of fibrosis in the Med and High combination arms.

Conclusion: Treatment with the THR-beta agonist TERN-501 in combination with the FXR agonist TERN-101 led to greater NAS and fibrosis improvements from baseline compared with single agent treatments, likely driven by increased anti-steatotic activity. These data suggest that combining the robust anti-steatotic effects of a selective THR-beta agonist with an FXR agonist may provide a superior therapeutic benefit for NASH over either agent alone. The use of AI-digital pathology can provide granularity in NASH drug development during the preclinical phase, which may be translated to current use in NASH clinical trials.

Combination therapy of TERN-501, a selective agonist of thyroid hormone receptor (THR) beta, with TERN-101, a farnesoid X receptor (FXR) agonist, improves nonalcoholic steatohepatitis (NASH) in the GAN diet-induced obese and biopsy-confirmed mouse model

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TERNS PHARMACEUTICALS

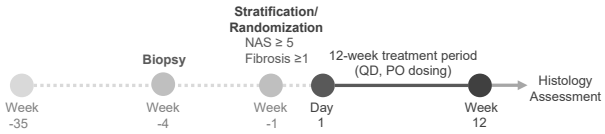
1 INTRODUCTION

- Nonalcoholic steatohepatitis (NASH) is a serious condition that may require a combination therapy to optimize disease resolution. TERN-501, a potent and selective agonist of thyroid hormone receptor (THR)-β, and TERN-101, a non-steroidal agonist of farnesoid X receptor (FXR), were tested alone and in combination in the Gubra-Amylin NASH (GAN) diet-induced obese (DIO) and biopsy-confirmed mouse model of NASH (Møllerhøj et al., 2022)

2 AIM

- The aim of this study was to assess the efficacy of TERN-501 and TERN-101, both individually and in combination, on liver disease following a 12-week treatment period in the biopsy-confirmed GAN DIO-NASH mouse model with hepatic fibrosis

3 STUDY OUTLINE



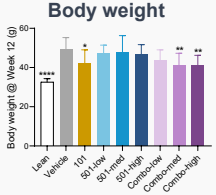
Group	Treatment	N	Model	Dose level (mg/kg)
1	Lean	10	Lean-chow	NA
2	Vehicle	16	DIO-NASH	NA
3	TERN-101	16	DIO-NASH	10
4	TERN-501-low	16	DIO-NASH	0.3
5	TERN-501-med	15	DIO-NASH	2
6	TERN-501-high	16	DIO-NASH	10
7	Combo-low	16	DIO-NASH	0.3 + 10
8	Combo-med	16	DIO-NASH	2 + 10
9	Combo-high	14	DIO-NASH	10 + 10

4 METHOD

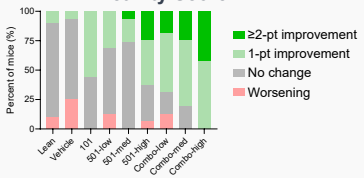
- Male C57BL/6J mice were fed the GAN diet high in fat, fructose, and cholesterol for 35 weeks. Liver biopsy was performed at week -4, and only animals with histology-confirmed NAFLD Activity Score (NAS) and fibrosis (i.e., steatosis =3, lobular inflammation ≥2; fibrosis stage ≥F1) as defined by Kleiner (2005) were included and stratified into treatment groups.
- TERN-101 (10 mg/kg, PO) and TERN-501 (0.3 [Low], 2 [Med], and 10 [High] mg/kg, PO) were administered once daily as single agents and in combination (n=14-16/group) for 12 weeks. Mice were kept on GAN diet throughout the study.
- Histological analyses were performed at baseline and end of treatment to assess NAS and fibrosis on H&E and Picro Sirius Red stained biopsies, respectively. Liver biopsies were also analyzed by stain-free artificial intelligence (AI)-based digital pathology (HistoIndex®) using two-photon excited fluorescence (TPEF) and second harmonic generation (SHG) to quantify steatosis and fibrosis, respectively

5 RESULTS

Body weight



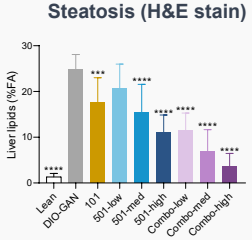
NAFLD Activity Score



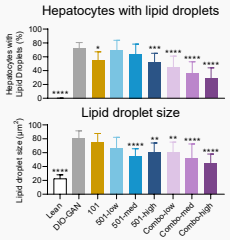
Left: Mean (±SD) body weight at Week-12. Statistical comparison to vehicle control determined by ANOVA followed by correction for multiple comparisons. *p<0.05, **p<0.01, ***p<0.0001.
Right: Change (pre-post) in NAFLD Activity Score (NAS) from baseline. Bars represent the percent of mice with categorical change in NAS.

- **TERN-501 monotherapy did not significantly impact body weight; TERN-101 and combination treatment resulted in modest decreases in body weight**
- **NAS improvements were seen with monotherapies, but combination treatment was more effective**

Steatosis (H&E stain)

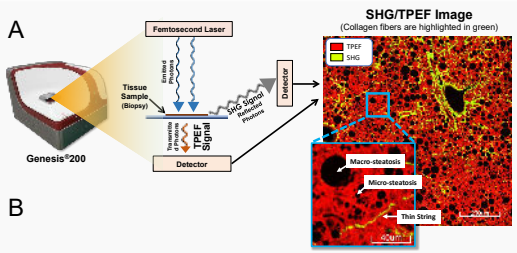


Hepatocytes with lipid droplets



Bars represent mean (±SD) at the end of study (n=10-16 mice per group) on liver steatosis fractional area (%FA, left), percent hepatocytes with lipid droplets (top right), and lipid droplet size (bottom right). Statistical comparison to vehicle control determined by ANOVA followed by correction for multiple comparisons. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

- **TERN-501 and TERN-101 monotherapies reduced steatosis, but combination treatment showed greater efficacy**

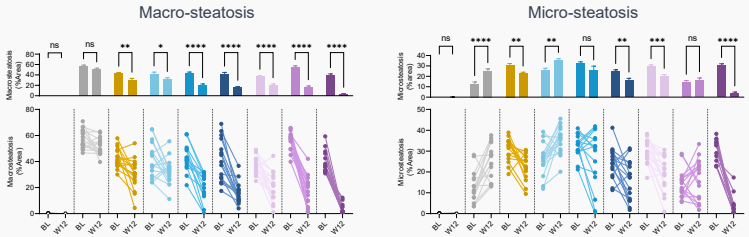


A) The Genesis® 200 system is a digital slide scanner that creates detailed images of unstained biopsy tissue. It uses a super-fast laser to excite the tissue at 780nm, then collects two types of signals: SHG at 390nm and TPEF at 550nm. These signals are colorized (SHG green, TPEF red) and combined to show liver structure and collagen fibers. SHG/ TPEF image showing fibrosis (green) and steatosis (black circles). Inset showing macro- (ø >12 μm) and micro- (ø ≤ 12 μm) steatotic vesicles by TPEF. Fibrosis thin string shown in green.

B) Left: correlation between steatosis measured from H&E-stained liver sections (Gubra, Lipid %FA) or unstained sections by TPEF microscopy (HistoIndex, %Area). Right: correlation between fibrosis measured from PSR-stained liver sections (Gubra, PSR %FA) or unstained sections by SHG microscopy (HistoIndex, %SHG). Dots represent individual mice at week-12 (steatosis), or baseline (fibrosis) colored by treatment group.

- **Strong correlation between stained and unstained methods for quantifying steatosis and fibrosis**

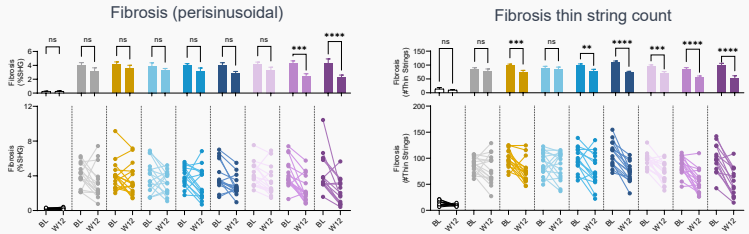
Steatosis (HistoIndex)



Bars represent mean (±SD) macro- (top left) or micro-steatosis (top right) percent area determined by TPEF microscopy across treatment groups. Before and after plots of individual mice for macro- (bottom left) and micro-steatosis (bottom right) at baseline (BL) and week-12 (W12). Statistical comparison to vehicle control determined by Two-Way ANOVA followed by correction for multiple comparisons. ns = not significant; ***p<0.001; ****p<0.0001

- **TERN-501 and TERN-101 significantly reduced both macro- and micro-steatosis as monotherapies but showed far greater efficacy when used in combination**

Fibrosis (HistoIndex)



Bars represent mean (±SD) fibrosis area (%SHG, top left) or thin string count (top right) in the perisinusoidal region determined by SHG microscopy across treatment groups. Before and after plots of individual mice for fibrosis area (bottom left) or thin string count (bottom right) at baseline (BL) and week-12 (W12). Statistical comparison to vehicle control determined by Two-Way ANOVA followed by correction for multiple comparisons.
ns = not significant; ***p<0.001; ****p<0.0001

- **Perisinusoidal fibrosis was significantly reduced with the combination of TERN-501 and TERN-101**
- **The number of fibrosis thin strings, defined as a fibrotic structure with a width:length ratio of ≤0.25, were significantly reduced by TERN-501 and TERN-101 as monotherapies but combination treatment showed greater efficacy. Such fine feature changes usually can only be reliably observed and reproduced with the stain-free method.**

6 CONCLUSIONS

- TERN-501 monotherapy showed robust anti-steatotic activity with some evidence of fibrosis improvement after 12-weeks of treatment in the GAN DIO-NASH mouse model
- Multiple efficacy endpoints, including NAS, steatosis, and fibrosis were significantly improved when TERN-501 was used in combination with the FXR agonist TERN-101
- These data suggest that combining TERN-501, a selective THR-β agonist, with the FXR agonist TERN-101 may lead to greater improvements in both steatosis and fibrosis in NASH over either agent alone
- Stain-free AI-digital pathology (HistoIndex) showed strong correlation with traditional stained histological analyses on both steatosis and fibrosis, and also enabled the assessment of finer morphological features
- The DUET study, a 12-week Ph2a trial fully enrolled and currently ongoing (NCT05415722), will evaluate the efficacy of TERN-501 administered alone and in combination with TERN-101 in patients with presumed non-cirrhotic NASH and fibrosis

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