Combination of TERN-101, a farnesoid X receptor agonist, and TERN-501, a selective agonist of thyroid hormone receptor beta, reduces activation of inflammatory and fibrotic gene pathways in a mouse model of non-alcoholic steatohepatitis

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1 • INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is a serious and progressive liver disease characterized by steatosis and liver inflammation with or without fibrosis. Currently there are no approved therapies for NASH.

FXR agonists can reduce steatosis, inflammation, and improve fibrosis, and are the most advanced class in development for the treatment of NASH. TERN-101 is a potent, nonsteroidal FXR agonist with enhanced liver distribution and is currently being evaluated in a Phase 2a clinical trial in NASH patients (NCT04328077).

THR-β agonists can increase metabolism, normalize blood lipid parameters, and profoundly reduce steatosis in NASH patients. TERN-501 is a potent (EC_{50}=100 nM) and selective THR-β agonist (23-fold THR-β vs. THR-α) that preferentially distributes to the liver.

Combinations of pharmacological agents will likely be necessary to achieve high response rates in NASH. In this study we explored the efficacy of combining TERN-101 with TERN-501 in a diet-induced obese (DIO) mouse model of NASH.

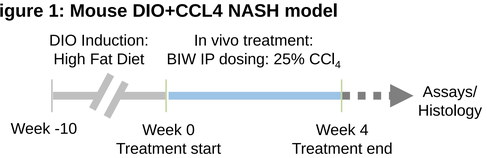
2 • AIM

To evaluate the efficacy of combining the FXR agonist TERN-101 with the THR-β agonist TERN-501 in a DIO mouse model of NASH

3 • METHODS

- Male C57BL/6J mice (8 individuals per dosing group) were fed a high fat diet for 10 weeks to induce obesity, followed by compound treatment (once daily oral [PO] via gavage) and 2x weekly IP injections of CCl_4 for 4 weeks. On day 28 of treatment, animals were euthanized for sample collections.
- Analysis of cholesterol, triglycerides, and ALT was done using a Hitachi 7180 clinical analyzer.
- Liver samples were processed for lipid quantification (colorimetric assays, SpectraMax 340PC384), histology, and RNA analysis.
- RNAseq library preparation (n=5 per group) and sequencing was performed using Illumina standard protocols. Alignment of sequencing reads was performed using STAR aligner and read counts were estimated using RSEM. Differentially expressed genes (DEGs) relative to NASH control were determined using EdgeR. Gene ontology analysis was performed using Advita software.

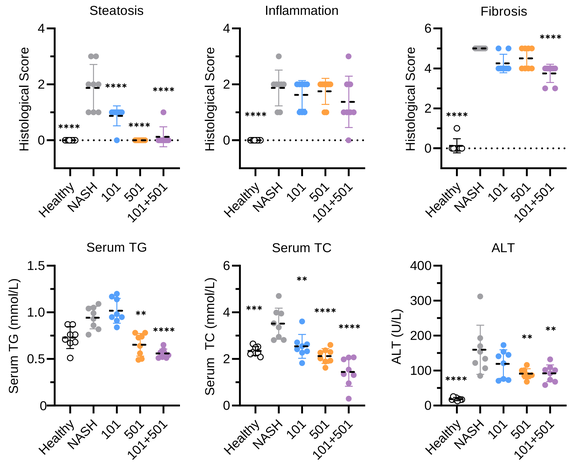
Figure 1: Mouse DIO+CCL4 NASH model



Group/Compound	Target	Dose	Route
Lean chow (healthy)*	NA	NA	NA
NASH control	NA	Vehicle†, QD	PO
TERN-101	FXR	3 mg/kg, QD	PO
TERN-501	THR-β	1 mg/kg, QD	PO
TERN-101+TERN-501	FXR+THR-β	3 mg/kg+1 mg/kg, QD	PO

4 • RESULTS

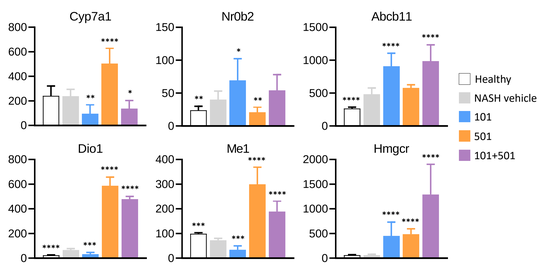
Figure 2: Efficacy of TERN-101 and TERN-501 alone and in combination in a mouse DIO+CCL4 NASH model



Liver steatosis (upper left), inflammation (upper middle) and fibrosis (upper right) were quantified by histological analysis for degree of steatosis, lobular inflammation, and fibrosis. Serum was collected at termination and analyzed for triglycerides (TG, lower left), total cholesterol (TC, lower middle) and a biomarker of liver damage, alanine aminotransferase (ALT, lower right). Data for individual animals (dots) and mean (dashed line) are presented; **p <0.01, ***p <0.001, ****p <0.0001 vs. NASH vehicle control (NASH). Statistics determined by one-way ANOVA followed by Tukey.

- The combination of TERN-101 and TERN-501 significantly improved multiple components NASH

Figure 3: Expression of FXR and THR-β target genes

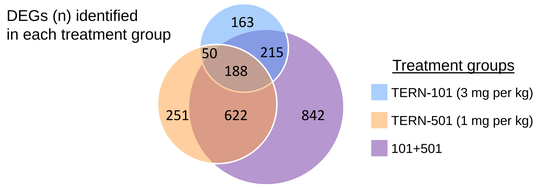


Mean (± SD) expression levels (CPM values) of genes associated with FXR and THR-β pathway activation determined by RNAseq. *p <0.05, **p <0.01, ***p <0.001, ****p <0.0001 vs. NASH vehicle control.

- FXR and THR-β pathway genes were modulated as expected in both single and combination treatment groups

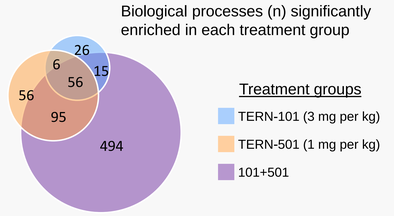
Figure 4: Differential expression analysis by RNAseq

- The combination treatment of TERN-101 and TERN-501 resulted in >800 unique differentially expressed genes (DEGs, purple circle)
- A significantly higher number of DEGs were observed in DIO NASH mice treated with the combination of TERN-101 and TERN-501 than expected based on single agent treatments alone



The number and overlap of DEGs relative to NASH control are shown for each treatment group using fold-change and adjusted p-value cutoffs of >1.5 and <0.05, respectively.

Figure 5: Gene ontology (GO) term enrichment analysis

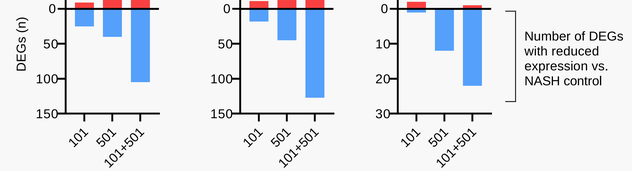


The number and overlap of biological processes that were significantly enriched in treatment groups relative to NASH control. An FDR-adjusted p-value of <0.05 was used as a cut-off for statistical significance.

- A significantly higher number of biological processes were enriched by the combination treatment of TERN-101 and TERN-501 (above, purple circle)
- Biological processes related to immune processes were preferentially enriched by the combination of TERN-101 and TERN-501 (Table 1)
- A greater number of DEGs associated with biological processes relevant to NASH were suppressed by the combination of TERN-101 and TERN-501 than by single agent treatments (right, blue bars)

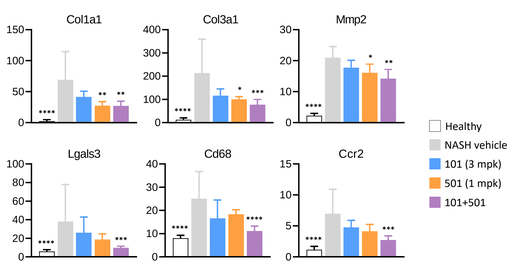
Table 1: Top immune-related processes significantly enriched only by the combination of TERN-101 and TERN-501

GO term	GO term ID	DEG Count (n)	Total Genes (n)	Corrected P-value
Immune response	0006955	216	941	1.21E-10
Inflammatory response	0006954	124	467	1.12E-09
Myeloid leukocyte activation	0002274	55	156	1.59E-08
Immune system process	0002376	327	1674	3.94E-08
Leukocyte activation	0045321	145	615	5.79E-08
Positive regulation of immune system process	0002684	156	687	1.86E-07
Leukocyte migration	0050900	69	233	2.33E-07
Regulation of immune response	0050776	132	567	5.75E-07
Regulation of immune system process	0002682	202	972	9.68E-07
Leukocyte activation involved in inflammatory response	0002269	18	32	5.1E-06



Number and expression change direction (blue: reduced expression vs. NASH control; red: increased expression vs. NASH control) of DEGs associated with selected GO terms.

Figure 6: Expression of genes associated with fibrosis and inflammation



Mean (± SD) expression levels (CPM values) of genes associated with fibrosis and inflammation pathways determined by RNAseq. *p <0.05, **p <0.01, ***p <0.001, ****p <0.0001 vs. NASH vehicle control.

- The combination of TERN-101 and TERN-501 significantly reduced expression of fibrosis and inflammatory genes

5 • CONCLUSIONS

- The combination of the FXR agonist TERN-101 and the THR-β agonist TERN-501 showed robust efficacy in a mouse DIO model of NASH by profoundly reducing steatosis and significantly improving fibrosis, serum TG, TC, and ALT
- The combination of TERN-101 and TERN-501 resulted in more DEGs than treatment with either TERN-101 or TERN-501 alone due to a higher number of down-regulated DEGs associated with biological processes relevant to NASH
- Together these results suggest that the combination of the FXR agonist TERN-101 and the THR-β agonist TERN-501 may provide additional benefits for NASH patients than either treatment alone

6 • CONTACT INFORMATION

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Disclosures: authors are employees/shareholders in Terns Pharmaceuticals, Inc.