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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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, non-nuclear FXR agonist with enhanced liver distribution and is currently being evaluated in a Phase 2a clinical trial in NASH patients (NCT04328977). THR-β agonists can increase metabolism, normalize bile lipid parameters, and profoundly reduce steatosis in NASH patients. TERN-501 is a potent (EC50=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.

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

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 2 weekly IP injections of CCl4, for 4 control. 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 340PC948), histology, and RNA analysis.
• RNAsseq 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 RSeQC. Differentially expressed genes (DEGs) relative to NASH control were determined using Edgar. Gene ontology analysis was performed using Avadna software.

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, liver inflammation, and fibrosis. Serum was collected at the end of treatment for triglyceride assay. To assess the potential for CCl4-induced liver damage, alanine aminotransferase (ALT; lower right). Data for individual animals (mean± standard deviation) are presented. *p < 0.05, **p < 0.01, ***p < 0.001 vs. NASH vehicle control (NASH). Statistical determination one-way ANOVA followed by Tukey.

Figure 3: Expression of FXR and THR-β target genes
The combination of TERN-101 and TERN-501 significantly improved multiple components NASH

Figure 4: Differential expression analysis by RNAsseq

- 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

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.

Figure 6: Expression of genes associated with fibrosis and inflammation
A significantly higher number of biological processes were enriched by the combination treatment of TERN-101 and TERN-501 (above, purple circle)

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

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

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<th>GO term</th>
<th>GO term ID</th>
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<th>TERN-501</th>
<th>TERN-101+501</th>
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