TERN-501, a potent and selective agonist of thyroid hormone receptor beta, strongly reduces histological features and biomarkers of non-alcoholic steatohepatitis associated pathology in rodent models

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INTRODUCTION

Liver inflammation and damage resulting from hepatic fat accumulation are key drivers in the progression of non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH). Selective agonism of thyroid hormone receptor beta (THR-beta) in the liver has been shown to markedly reduce liver fat, hepatic inflammation, and damage raising the prospect of an efficacious NASH treatment in the future.[1]

AIM

The aim of the study was to investigate the potency and selectivity of TERN-501 in a biochemical assay and to assess the translation into efficacy and safety in rodent models capable of measuring THR-beta agonism.

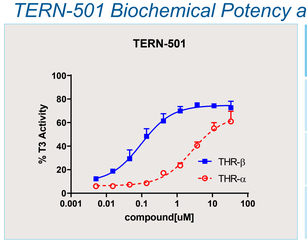
METHODS

The potential of TERN-501 to become a therapeutic agent for the treatment of NASH was established in multiple pre-clinical settings

- The ability of TERN-501 to selectively agonize THR-beta was assessed biochemically using THR-beta or THR-alpha / RXR heterodimeric assays.[2]
- Male SD rats (4 individuals per dosing group) were fed a cholesterol enriched, otherwise normal, diet (1.5% cholesterol & 0.5% cholic acid) for 14 days. Compound(s) were administered via the intraperitoneal route (IP). Blood was collected at t = 0 (pre-treatment) and t = 24 hrs. Serum levels of cholesterol and triglycerides (TG) were determined for these two time points. Additionally, t = 6 hours plasma samples were prepared and analyzed to confirm test article exposure.
- Male C57BL/6J mice (8 individuals per dosing group) were fed a high fat diet (HFD) for 10 weeks. Then compound treatment (once daily oral [PO] via gavage) and 2x weekly IP injections of CCl_4 for 4 weeks was initiated. On day 28 of treatment, animals were euthanized for brain, heart and liver weight measurement and blood and liver sample collections.
- Cholesterol, triglycerides, ALT; Analysis of these parameters was done in a Hitachi 7180 clinical analyzer.
- Liver samples were processed for lipid quantification (colorimetric assays, SpectraMax 340PC384), histology, and RNA analysis (RT-qPCR and RNAseq).

RESULTS

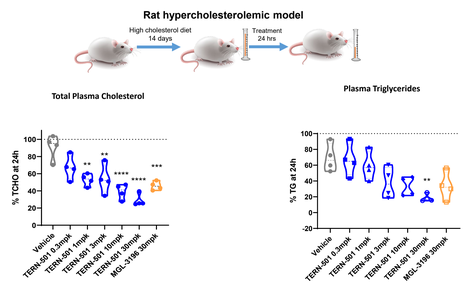
TERN-501 Biochemical Potency and Selectivity



TERN-501	
EC_{50} THR-beta	0.1 microM ±0.05 microM
EC_{50} THR-alpha	2.5 microM ± 1.5 microM
THR-beta/THR-alpha	23# ±5.8

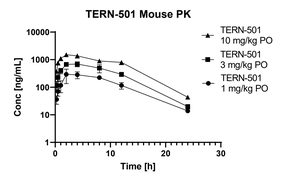
Blue curve THR-beta; red curve THR-alpha. # T3 normalized. Table data are average and SD from multiple assays (n=38) Selectivity (THR-beta/alpha) for MGL-3196 was 15 ± 3.7 (lit: 28)[2]. Selectivity (THR-beta/alpha) for VK2809A[3] was 2.

TERN-501 Highly Effective in Rat Hypercholesterolemic Model[3]



Total plasma cholesterol (left) and plasma triglycerides (right) were quantified in the plasma of cholesterol-fed SD rats at t = 24 hrs after drug administration (IP). Data are displayed as % of value measured relative to t = 0. Data for individual animals are shown. Mean value displayed via dashed line ; **p< 0.01, ***p< 0.001 , ****p< 0.0001 vs Vehicle Control; statistics determined by one-way ANOVA followed by Tukey.

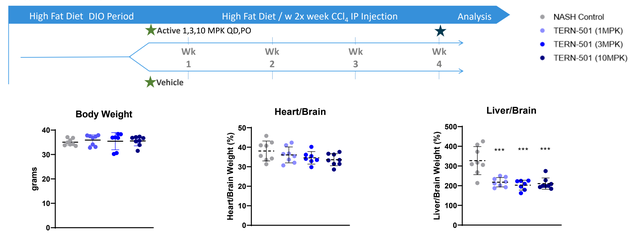
TERN-501 Mouse PO PK



AUC [ng/mL*h]	
1 mg/kg	3,320
3 mg/kg	7,940
10 mg/kg	17,800

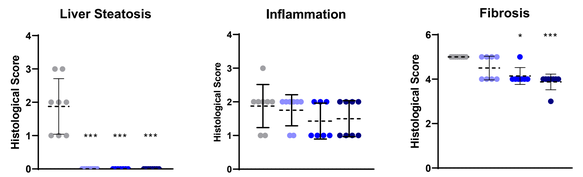
Mice (male C57 BL6, non-fasted, 3 animals/time point) were dosed with TERN-501 via oral gavage. Plasma was collected at the indicated time points and TERN-501 levels were quantified via LC-MS/MS method.

Mouse NASH Model and TERN-501 Effects on Body and Organ Weight



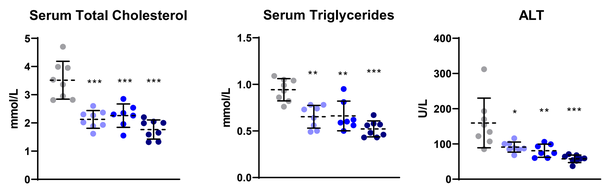
Schematic description of study design and display of body weight (left), heart/brain ratio (middle) and liver/brain ratio (right) at the end of the study. Data for individual animals (dots) and mean (dashed line) are depicted. Statistical significance for liver/brain ratio change of any treatment group was ***p<0.001 vs NASH Control (Vehicle); statistics determined by one-way ANOVA followed by Tukey.

Liver Histology: TERN-501 Effects on Liver Steatosis and Fibrosis in Mouse NASH Model



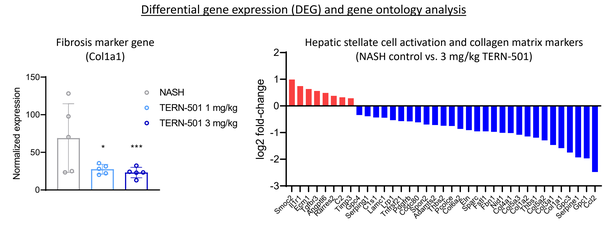
Liver steatosis (left), inflammation (middle) and fibrosis (right) were quantified by histological analysis of degree of steatosis (Score 0, <5%; 1, 5-33%; 2, 34-66%; 3, >66%), lobular inflammation (Score 0, none; 1, <2 foci per 20X field; 2, 2-4 foci per 20X field; 3, >4 foci per 20X field) and fibrosis (0-6; Score 0, none; 1, perisinusoidal mild; 2, perisinusoidal moderate; 3, portal / periportal; 4, perisinusoidal and portal/periportal; 5, bridging fibrosis; 6, cirrhosis). Data for individual animals (dots) and mean (dashed line) are presented ; *p< 0.05, ***p< 0.001 vs NASH Control (Vehicle); statistics determined by one-way ANOVA followed by Tukey.

Serum Analysis: TERN-501 Effects on Lipids and Indicators of Liver Injury (ALT) in Mouse NASH Model



Serum was collected at termination and analyzed for total cholesterol (left), triglycerides (middle) and a biomarker of liver damage, alanine aminotransferase (ALT, right) using an automatic biochemical analyzer. Data are presented as Mean ± SD (n=8); *p< 0.05,**p<0.01 ***p< 0.001 vs NASH Control (Vehicle); statistics determined by one-way ANOVA followed by Tukey.

RNAseq Analysis: Gene Expression of Collagen Matrix Markers & Stellate Cell Activation with TERN-501 in Mouse NASH Model



Liver whole transcriptome analysis was performed using RNAseq of NASH control (Vehicle) and TERN-501 (1 and 3 mg/kg) treated animal groups (n=5 animals per group). Collagen type 1 alpha 1 chain (Col1a1) gene expression and significance (adjusted p-value) in control and TERN-501 treatment groups is shown as a representative marker of fibrosis (left). Waterfall plot of differentially expressed genes (adjusted p-value < 0.05) associated with collagen extracellular matrix and hepatic stellate cell activation in NASH vehicle vs TERN-501 (3 mg/kg) treatment groups (right). *p-value<0.05; ***p-value<0.001

CONCLUSIONS

TERN-501 is a potent and selective THR-beta agonist. TERN-501 reduced serum cholesterol levels in a hypercholesterolemic rat model and significantly reduced liver steatosis, fibrosis, and serum markers of liver damage in a NASH mouse model. These results support further investigation of TERN-501 as a potential treatment for NASH.

ACKNOWLEDGEMENTS

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