A novel farnesoid X receptor agonist, TERN-101, reduces liver steatosis, inflammation, ballooning and fibrosis in a murine model of non-alcoholic steatohepatitis

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1 • INTRODUCTION

The Farnesoid X Receptor (FXR) is a nuclear hormone receptor that controls the conversion of cholesterol into bile acids and maintains homeostasis of multiple metabolic pathways including lipid metabolism and glucose homeostasis.

Activation of the FXR pathway using synthetic FXR agonists may help control metabolic disorders such as non-alcoholic steatohepatitis (NASH). Intercept Pharmaceuticals reported top line Ph3 clinical results of obeticholic acid (OCA) with OCA showing superiority in the proportion of F2/F3 participants with at least 1 stage of fibrosis improvement without worsening of NASH.

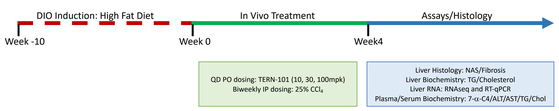
TERN-101, a novel non-steroidal agonist of FXR, has entered early stage clinical trials.

2 • AIM

To evaluate the efficacy and mechanism of action of TERN-101 in a mouse model of NASH

3 • METHOD

- EC_{50} values for FXR were determined by a fluorescence-based FXR coactivator assay. Half-log serial dilutions of TERN-101 or OCA (10μM-3nM) were incubated with human FXR ligand binding domain produced in Sf9 insect cells, labeled coactivator SRC-1 peptide and buffer for 1h at 25ºC. TGR5 activity was measured using a cell-based cAMP assay. Half-log serial dilutions of TERN-101 or OCA (10μM-3nM) were added to Chinese Hamster Ovary cells expressing recombinant human TGR5. After 30min at RT, cAMP was measured using an HTRF readout.
- EC_{50} values for FXR-regulated gene expression were determined using a cell-based RNA assay. Half-log serial dilutions of TERN-101 or OCA (3μM-3nM) were added to human HuH7 hepatoma cells. After 11h at 37ºC, RNA was isolated and analyzed by RT-qPCR using primers to small heterodimer partner (SHP), bile salt export pump (BSEP) and fibroblast growth factor 19 (FGF-19).
- Male C57/BL6J mice were fed a high fat diet (D12492, Research Diet, fat/protein/carbohydrate 60/20/20 Kcal%, 10w) to induce obesity (>36g mouse) prior to daily oral TERN-101 and biweekly intraperitoneal carbon tetrachloride (CCl_4) treatment for 4 weeks.



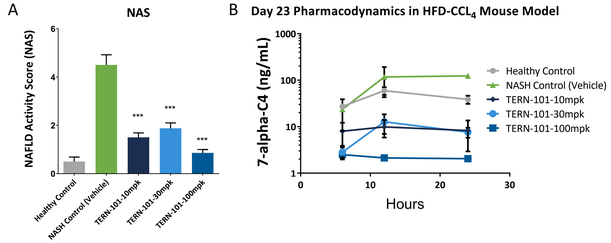
4 • RESULTS

Table 1. TERN-101 is a potent and selective FXR agonist

Assay	TERN-101 EC_{50} (nM)	OCA EC_{50} (nM)
FXR Agonist	57	73
TGR5 Agonist	>10,000	770
SHP Gene Induction/HuH7	50	200
BSEP Gene Induction/HuH7	40	200
FGF-19 Gene Induction/HuH7	30	130

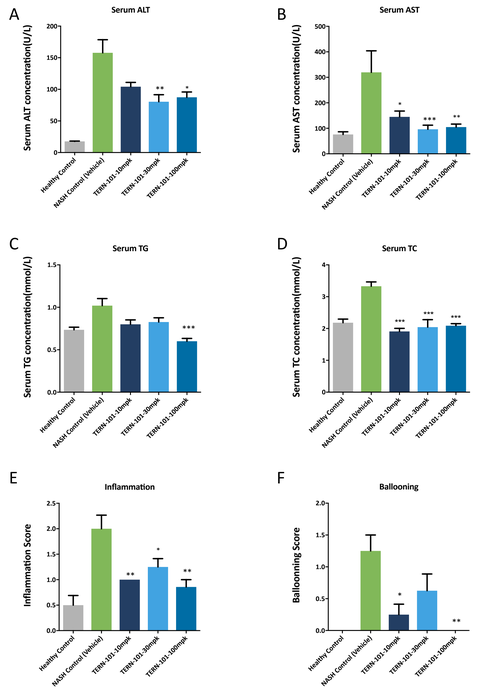
4 • RESULTS

Figure 1. TERN-101 reduces NAFLD Activity Score (NAS) and 7α-Hydroxy-4-cholesten-3-one (7-α-C4) in HFD/CCl_4 NASH mice



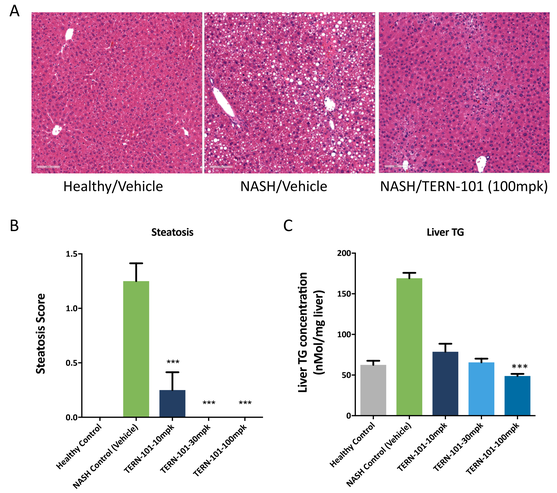
(A) NAS was determined by analysis of liver tissue histology using H&E stain. Liver steatosis, inflammation and ballooning were quantified by histological analysis of degree of steatosis (Score 0, <5%; 1, 5-33%; 2, >33-66%; 3, >66%), inflammation (Score 0, none; 1, <2 foci per 200X field; 2, 2-4 foci per 200X field; 3, >4 foci per 200X field) and ballooning (Score 0, none; 1, few ballooning hepatocytes; 2, many hepatocytes with prominent ballooning). Data are presented as Mean ± SEM (n=7 for TERN-101 100 mpk, n=8 for all other groups; ***p< 0.001 vs NASH Control (Vehicle); statistics determined by one-way ANOVA followed by Tukey). (B) Plasma was collected at 6, 12 and 24 h post TERN-101 dosing on day 23 and analyzed for 7a-Hydroxy-4-Cholesten-3-one (7-α-C4) using LCMS. Data are presented as Mean ± SD (n=3 for the 6 and 12 h timepoints, n=2 for the 24 h timepoint).

Figure 2. TERN-101 reduces serum biomarkers of liver damage, triglycerides and cholesterol and liver inflammation and ballooning in HFD/CCl_4 NASH mice



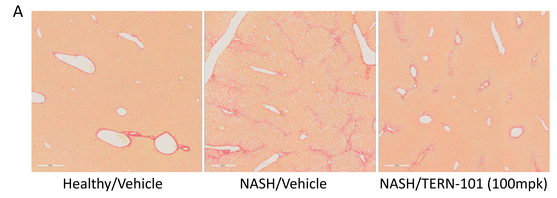
Serum at termination was analyzed for (A) alanine aminotransferase (ALT), (B) aspartate aminotransferase (AST), (C) triglyceride and (D) total cholesterol levels using an automatic biochemical analyzer. (E) Liver inflammation and (F) ballooning were quantified by histological analysis as described in Figure 1A. Data are presented as Mean ± SEM (n=7 for TERN-101/100mpk, n=8 for all other groups; ***p<0.001, **p<0.01, *p<0.05 vs NASH Control (Vehicle); statistics determined by one-way ANOVA followed by Tukey).

Figure 3. TERN-101 reduces liver steatosis and triglycerides in HFD/CCl_4 NASH mice



(A) Liver steatosis was analyzed using H&E stain on liver sections. Representative histology is shown (lean healthy control, NASH vehicle control and NASH with 100mpk TERN-101 shown). (B) Liver steatosis was quantified by histological analysis as described in Figure 1A. (C) Liver triglyceride was measured from liver tissue using an automatic biochemical analyzer. Data are presented as Mean ± SEM (n=7 for TERN-101/100 mpk, n=8 for all other groups; ***p<0.001, **p<0.01, *p<0.05 vs NASH Control (Vehicle); statistics determined by one-way ANOVA followed by Tukey).

Figure 4. TERN-101 reduces liver fibrosis in HFD/CCl_4 NASH mice



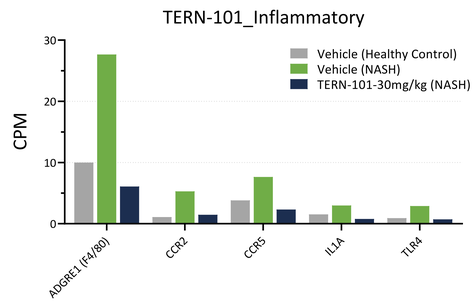
(A) Liver tissue fibrosis was analyzed using Sirius Red stain. Representative histology is shown (lean healthy control, NASH vehicle control and NASH with 100mpk TERN-101 shown). (B) Liver fibrosis was quantified by histological analysis of the percentage of Sirius Red-positive liver sections. (C) Liver RNA was analyzed using RT-qPCR with primers for mouse COL1A1 . Data are presented as Mean ± SEM (n=7 for TERN-101/100 mpk, n=8 for all other groups; ***p<0.001, *p<0.05 vs NASH Control (Vehicle); statistics determined by one-way ANOVA followed by Tukey).

Figure 5. TERN-101 regulates liver gene expression in HFD/CCl_4 NASH mice including FXR-target, inflammatory and fibrosis genes

A

	Gene	TERN-101 (30mg/kg) Relative to Vehicle (NASH)
FXR-Target Genes	SHP	4.6
	BSEP	5.1
	OST-B	135.7
	CYP7A1	0.02
	CYP8B1	0.007

B



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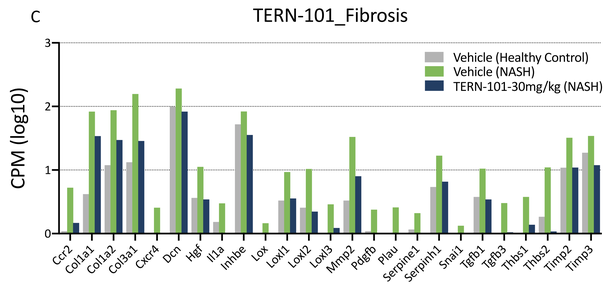

Liver RNA was isolated at termination on day 28 and analyzed by RNAseq. Inflammatory and fibrosis system signatures were defined using "Reactome" program.

5 • CONCLUSIONS

- TERN-101 is a potent and selective non-steroidal FXR agonist.
- TERN-101 strongly suppressed liver steatosis, inflammation, ballooning and fibrosis in a diet-induced obese mouse model of NASH.
- Inflammatory and fibrosis RNA signatures were reduced by TERN-101 in this mouse NASH model.

6 • DISCLOSURES/CONTACT

Authors are employees/stockholders in Terns Pharmaceuticals

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