Pharmacokinetics, Tissue Distribution and Pharmacodynamics of TERN-101, A Novel Farnesoid X Receptor (FXR) Agonist, in Preclinical Species

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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.[1-3]
- FXR is an important clinical target for non-alcoholic steatohepatitis (NASH), validated by the positive Phase 3 results of obeticholic acid (OCA)[4], a steroidal FXR agonist that activates FXR through binding of bile acid receptors.
- TERN-101, a non-steroidal, liver-enriched FXR agonist currently in Phase 1 clinical trials, was assessed in preclinical species for its pharmacokinetics (PK), tissue distribution, and pharmacodynamics (PD).

METHODS

In vitro

- The rate of hepatic metabolism of TERN-101 (1 μM) was assessed in cryopreserved mouse, rat, dog, monkey, and human hepatocytes (0.5x10^6 cells/mL) at 37°C for 2h. In vitro half-life values were determined and then scaled to predict hepatic clearance using the well-stirred liver model with no correction for plasma protein binding[5].
- The *in vitro* uptake test system for transporters OATPs (1B1 and 1B3) used a polarized monolayer of MDCK-II cells grown on permeable support. The MDCK-II cells were treated to express the transporter of interest or a control vector. TERN-101 was tested at 1 μM , 3 μM (with and without 100 μM rifampin), and 10 μM.

In vivo

- TERN-101 PK in Sprague Dawley (SD) rats, beagle dogs, and cynomolgus monkeys were determined following an intravenous (IV) bolus and oral administrations of TERN-101. Serial blood samples (0-24h) were collected for plasma PK.
- TERN-101, cilofexor, and tropifexor tissue distribution in SD rats (n=3 rats/compound) was determined following a 30-minute IV infusion at 2 mg/kg for each compound. Blood, liver, kidney, and lung tissue samples were collected at 2h post dose to determine tissue/plasma ratio.
- [14-C]TERN-101 tissue distribution (n=10 rats) was determined in pigmented Long Evans rats following an oral dose of TERN-101 at 5 mg/kg (100 μCi/kg of [14-C]TERN-101). Tissue samples were collected up to 168h.
- TERN-101 PK/PD profiles were determined in cynomolgus monkeys via oral administration of TERN-101 suspension at 0 (vehicle), 0.3, 1, and 5 mg/kg. Blood samples (0-24 hours) were collected for plasma PK and PD measurements. TERN-101 (1 mg/kg) was orally dosed for 7 consecutive days in cynomolgus monkeys (n=6) to determine its PK/PD effects following multiple doses.
- For mouse PD/MOA experiments, C57BL/6 mice (n=6/cohort) were given a single oral dose of vehicle, TERN-101 (10 mg/kg), or OCA (30 mg/kg). Tissue RNA was collected at 6h post-dose and analyzed by RT-qPCR and RNAseq. For RT-qPCR, gene-specific primers were used to quantitate FXR-regulated gene expression in liver and ileum using the 2-ddCT method. For RNAseq analysis, mRNA was extracted from total liver and sequenced using standard Illumina library preparation and sequencing protocols. Differentially expressed genes were determined using RSEM and edgeR software packages and analyzed using Advaita Bio's iPathwayGuide software.

RESULTS

In Vitro DMPK

TERN-101 In Vitro Metabolic Stability in Hepatocytes

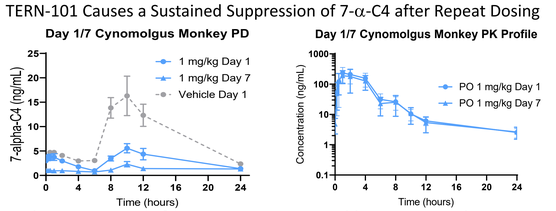
Species	t½ (min)	In vitro Metabolic CL_{pred} (L/h/kg) [a,b]	Hepatic Extraction (%)
Mouse	43.6 ± 2.83	4.36 ± 0.06	80.7 ± 1.02
Sprague-Dawley Rat	131 ± 4.11	1.57 ± 0.03	47.3 ± 0.78
Beagle Dog	126 ± 15.5	1.32 ± 0.05	71.0 ± 2.49
Cynomolgus Monkey	63.4 ± 0.78	1.68 ± 0.01	64.4 ± 0.28
Human	84.1 ± 6.48	0.83 ± 0.22	67.0 ± 1.73

a: Cryopreserved pooled mouse, rat, dog, monkey, and human hepatocytes were used; 2 hours incubation with TERN-101 concentration at 1 μM.
b: *In vitro* predicted hepatic clearance was not corrected for plasma protein binding.

- TERN-101 was moderately metabolized in hepatocytes of all species tested.

In Vivo DMPK

TERN-101 Plasma PK Profiles in Preclinical Species



TERN-101 PK Parameters in Preclinical Species

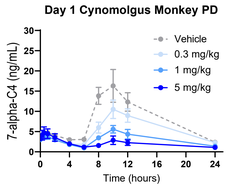
Species	CL (L/h/kg)	V_{dss} (L/kg)	IV Terminal $t_{1/2}$ (h)	Oral Bioavailability (%)
Sprague-Dawley Rat	2.55	1.31	2.45	21[a]
Beagle Dog	0.54	1.92	5.67	82[b]
Cynomolgus Monkey	0.30	0.60	1.32	18[c]

a: 10%DMSO, 10%Cremophor EL, and 80% (10%HP-β-CD)
b: Suspension of 1% Carboxymethyl cellulose, 0.25% Tween80, and 0.05% antifoam in water
c: 10% solutol, 20% PEG400, 0.5% Tween80, and 69.5% DI water

- TERN-101 has low to moderate clearance *in vivo*; the volume of distribution (V_{dss}) of TERN-101 is greater than the volume of total body water (0.70 L/kg) in rat and dog, smaller V_{dss} in monkeys is correlated with higher plasma protein binding.

TERN-101 PK/PD Profile in Cynomolgus Monkeys

Single Oral Dose of TERN-101 Causes a Dose-Dependent Reduction in 7-α-C4



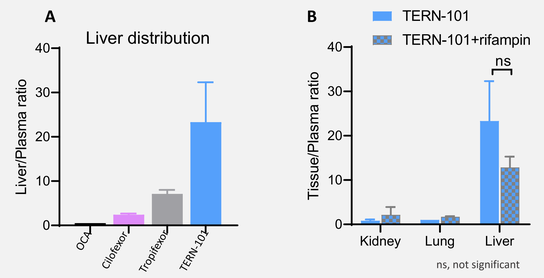
TERN-101	PK Parameters AUC_{0-24} (ng*hr/mL)	C_{max} (ng/mL)	T_{max} (hr)
0.3 mg/kg	196 ± 64	58.8 ± 30.2	2.17 ± 1.47
1 mg/kg	1000 ± 419	257 ± 124	1.83 ± 1.17
5 mg/kg	2720 ± 1500	709 ± 458	2.25 ± 1.47

TERN-101 Causes a Sustained Suppression of 7-α-C4 after Repeat Dosing

- Plasma exposure of TERN-101 was comparable on Day 1 and Day 7
- Sustained suppression of PD biomarker 7-α-C4 was achieved after repeated oral dosing

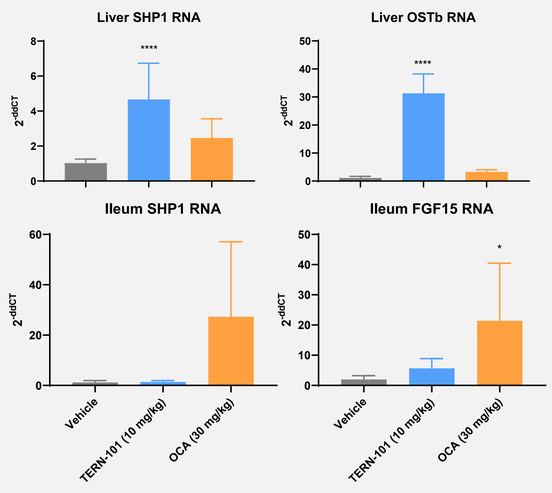
TERN-101 Tissue Distribution

TERN-101 Preferentially Distributes to Liver in Sprague Dawley Rats



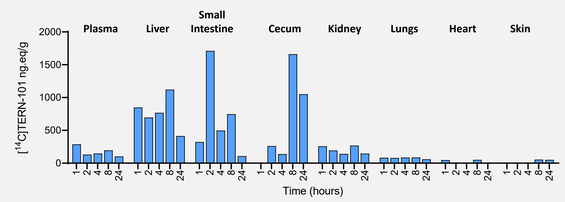
A. Liver to plasma distribution of TERN-101 compared to steroidal (OCA)[6] and non-steroidal (cilofexor and tropifexor) FXR agonists. **B.** Relative tissue to plasma distribution of TERN-101 in kidney, lung, and liver. TERN-101 concentration is approximately 20-fold higher in liver than in other organs. *In vitro* and *in vivo* experiments with rifampin, an inhibitor of OATP 1B1 and 1B3, show that TERN-101 is not an OATP 1B1/1B3 substrate.

TERN-101 Preferentially Induces FXR-Specific Genes in the Liver of Mice



Liver (top) and ileum (bottom) gene expression were analyzed using RT-qPCR. Data are presented as mean ± SD (n=6). Statistics (vs. vehicle) determined by one-way ANOVA followed by Tukey. *p < 0.05; ****p < 0.0001

Tissue Distribution of [14-C]TERN-101 and Metabolites in Long Evans Rats

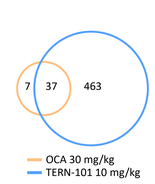


- The tissues with the highest radioactivity concentrations were the liver, small intestine, and cecum

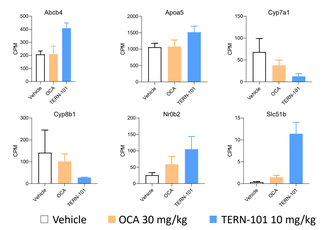
TERN-101 Liver Gene Expression and Pathway Analysis

TERN-101 Treatment Modulates Significantly More Genes and Pathways Relevant to NASH Compared to OCA

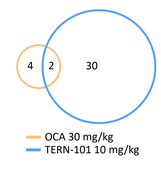
A Differentially expressed genes



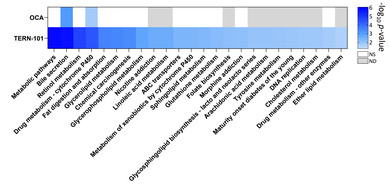
B Select FXR-related genes



C Global pathway enrichment



D Top 25 global pathways enriched by TERN-101 treatment



A. Differentially expressed genes relative to vehicle treated mice (fold-change ≥1.5; *q*-value <0.05). **B.** Average expression (CPM values) of FXR-related genes in vehicle, OCA (30 mg/kg), and TERN-101 (10 mg/mg) treated mice (n=6 per group). **C.** Global pathways significantly enriched in OCA and TERN-101 treatment groups. **D.** Top 25 most significantly enriched pathways in TERN-101 treatment group rank-ordered by statistical significance.

CONCLUSIONS

- TERN-101 preferentially distributed to the liver and exhibited high liver/plasma ratio in rodent species, approximately 3 to 20-fold higher than other investigational FXR agonists being studied for the treatment of NASH.
- The preferential liver distribution of TERN-101 is supported by preclinical PK/PD studies in mice; sustained suppression of 7- α-C4 observed in cynomolgus monkeys after repeat dosing suggests robust target engagement.
- RNAseq analysis of livers from mice treated with TERN-101 showed a more robust modulation of FXR-related genes and metabolic pathways relevant to non-alcoholic fatty liver disease compared to OCA treatment.
- TERN-101 exhibited low to moderate *in vivo* clearance, moderate volume of distribution, and oral bioavailability in the preclinical species tested. Biliary excretion was identified as the major elimination pathway for TERN-101.
- Taken together, the PK/PD profile of TERN-101 in preclinical species supports advancement into clinical trials and suggests TERN-101 has differentiated tissue distribution, and PD effects compared to other FXR agonists being studied for the treatment of NASH.

DISCLOSURE / CONTACT

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