Anti-inflammatory and anti-fibrotic activity of TERN-201, a semicarbazide-sensitive amine oxidase inhibitor, in a rat choline-deficient high-fat diet non-alcoholic steatohepatitis model

K. Klucher, C. Jones, Z. Zhou and M. Fenaux
Terns Pharmaceuticals, Foster City, California, USA

INTRODUCTION

Semicarbazide-Sensitive Amine Oxidase (SSAO, also known as vascular adhesion protein 1 [VAP-1]) is an ectoenzyme catalyzing the oxidative deamination of primary amines to aldehyde and hydrogen peroxide (H_2O_2), increasing systemic oxidative stress and damage to vasculature. In addition, a membrane-bound form of SSAO is upregulated on endothelial cells at sites of local injury enhancing leukocyte entry into affected tissue, thereby mediating inflammation.

Serum SSAO is increased in patients with histologically confirmed NASH and independently associated with fibrosis stage (Weston et al.). In rodent models, SSAO deficiency attenuates CCl_4-induced hepatic fibrosis and SSAO knockout mouse liver exhibits reduced steatosis and fibrosis when fed a western lifestyle diet (Weston et al.).

Pharmacological SSAO inhibition is anticipated to reduce inflammation and fibrosis in NASH patients. This novel mechanism provides an appealing opportunity to treat NASH, a disease with significant unmet medical needs. TERN-201, a novel, selective SSAO inhibitor, is currently in Phase 1 clinical trials.

AIM

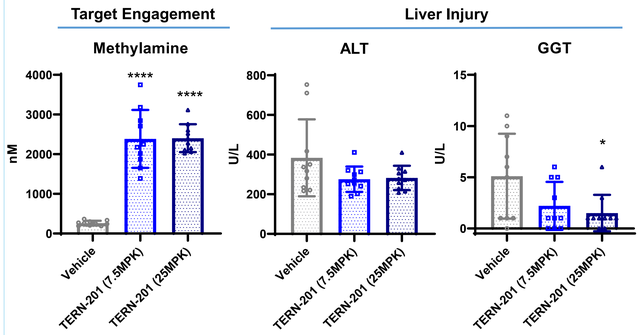
To evaluate the efficacy and mechanism of action of TERN-201 in a rat choline-deficient high-fat diet non-alcoholic steatohepatitis model.

METHODS

- Male Wistar rats were fed a choline-deficient high fat diet (CDHFD) for 4 weeks followed by tri-weekly intraperitoneal sodium nitrite ($NaNO_2$) treatment to induce fatty liver disease and fibrosis, respectively. TERN-201 dosing was administered for 8-weeks concomitant with the start of $NaNO_2$ treatment.
- Plasma samples were used for quantification of methylamine, AST, ALT, GGT, triglycerides, and cholesterol. Analysis of these parameters was done by HPLC-MS/MS (methylamine was derivatized to N-methylbenzamide for detection) or in a respons®910 clinical analyzer (remaining).
- Liver samples were processed for lipid quantification (respons®910 clinical analyzer), histology, and RNA analysis (RT-qPCR and RNAseq).

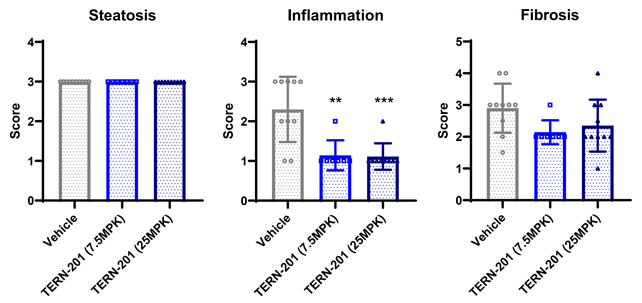
RESULTS

Plasma Biomarkers-TERN-201 Induces Methylamine and Reduces GGT



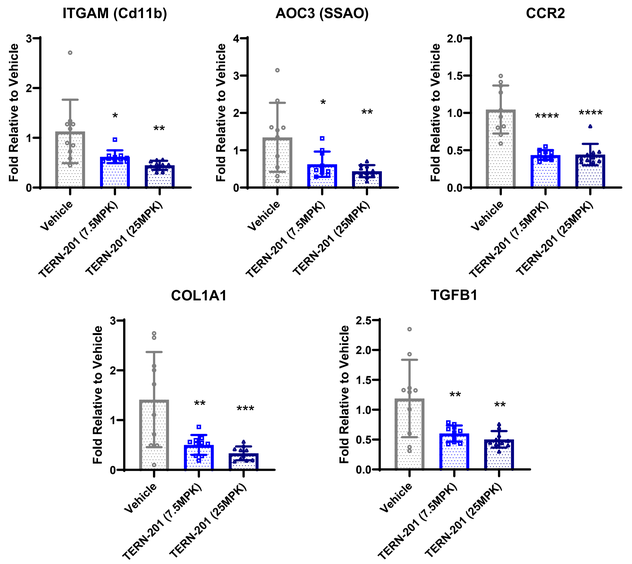
Plasma was collected at termination and analyzed for a biomarker of SSAO inhibition, methylamine, using LCMS (left) and two biomarkers of liver injury, alanine aminotransferase (ALT, middle) and Gamma-Glutamyl Transferase (GGT, right) using an automatic biochemical analyzer. Data are presented as mean ± SD (n=10); *p< 0.05, ****p< 0.0001 vs NASH control (Vehicle); statistics determined by one-way ANOVA followed by Tukey.

Liver Histology-TERN-201 Reduces Liver Inflammation



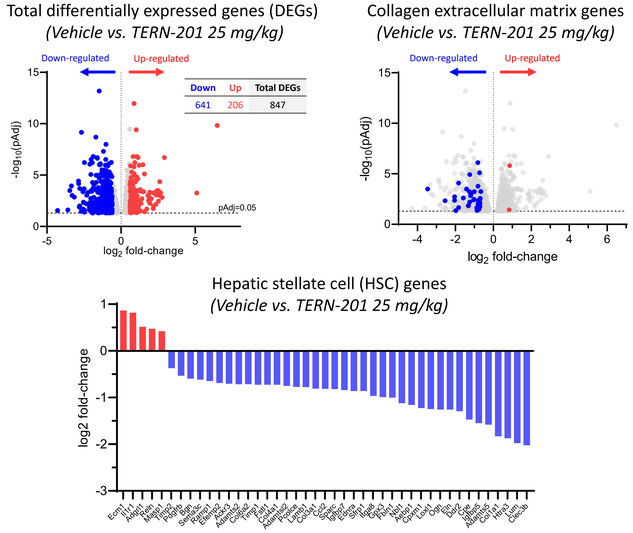
Liver steatosis (left), inflammation (middle) and fibrosis (right) were quantified by histological analysis of degree of steatosis (Score 0, <5%; 1, 5-33%; 2, 34-66%; 3, >66%), lobular inflammation (Score 0, none; 1, <2 foci per 20X field; 2, 2-4 foci per 20X field; 3, >4 foci per 20X field) and fibrosis (Score 0, none; 1, perisinusoidal or periportal; 2, perisinusoidal and portal/periportal; 3, bridging fibrosis; 4, cirrhosis). Data are presented as mean ± SD (n=10 for vehicle, n=7 TERN-201 @ 7.5mpk, n=9 for TERN-201 @ 25mpk); **p< 0.005, ***p< 0.001 vs NASH Control (Vehicle); statistics determined by one-way ANOVA followed by Tukey.

Liver RNA (RT-qPCR)-TERN-201 Reduces Genes Associated with Liver Inflammation and Fibrosis



Liver RNA was analyzed using RT-qPCR with primers for genes associated with inflammation (top) and fibrosis (bottom). Data are presented as mean ± SD (n=10); *p< 0.05, **p< 0.005, ***p< 0.001, ****p< 0.0001 vs NASH Control (Vehicle); statistics determined by one-way ANOVA followed by Tukey.

Liver RNA (RNAseq)-TERN-201 Reduces Genes Associated with Liver Collagen Extracellular Matrix and Hepatic Stellate Cell Biology

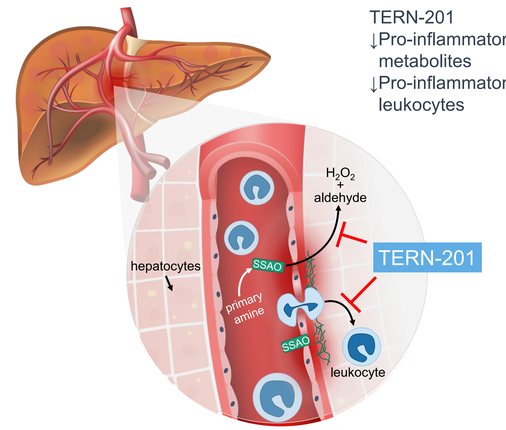


Liver whole transcriptome analysis was performed using RNAseq on the NASH control (Vehicle) and TERN-201 (25mpk) cohorts. The total number of differentially expressed genes (DEGs) is shown in the upper left panel (fold-change >1.5-fold, p value<0.01). Gene ontology/pathway enrichment analysis identified down-regulated genes associated with collagen extracellular matrix and hepatic stellate cell activation (Xiong et al.) in mice treated with TERN-201.

CONCLUSIONS

- TERN-201 strongly inhibited liver inflammation and inflammatory gene expression in a rat model of NASH.
- TERN-201 also reduced markers of fibrosis and hepatic stellate cell activation, supporting the further development of this drug in patients with NASH.

MODEL OF TERN-201 MOA



ACKNOWLEDGEMENTS

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REFERENCES

C. J. Weston et al., Vascular adhesion protein-1 promotes liver inflammation and drives hepatic fibrosis. J Clin Invest. (2015) 125:501-20.
Xiong X. et al., Landscape of intercellular crosstalk in healthy and NASH liver revealed by single-cell secretome gene analysis. Mol Cell. (2019) 75(3):644-660

CONTACT INFORMATION

Kevin Klucher, Terns Pharmaceuticals
1065 E. Hillsdale Blvd., Suite 100
Foster City, CA 94404
kklucher@ternspharma.com