Sustained FXR agonist M480 shows superior efficacy to THRβ agonist MGL-3196 in reversing NASH and advanced fibrosis

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INTRODUCTION

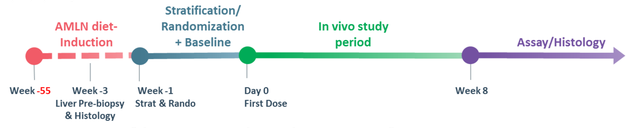
Sustained farnesoid X receptor (FXR) activation and thyroid hormone receptor beta (THRβ) modulation have both shown efficacy in clinical trials for non-alcoholic steatohepatitis (NASH); however, only FXR activation has shown fibrosis improvement.

AIMS

The aim of this study was to compare the efficacies of FXR and THRβ agonists in a diet-induced obese NASH model with advanced fibrosis and a chemically induced model of fibrosis.

MATERIAL & METHODS

To induce diet-induced obesity (DIO) driven NASH with advanced fibrosis, male C57BL/6 mice were fed a diet high in trans-fat, fructose and cholesterol (AMLN diet) for 52 wks. Only mice with biopsy-confirmed steatosis (score ≥2) and fibrosis (stage 3) were included. NASH and fibrosis efficacy were assessed by histological evaluation after 8 wks of treatment with sustained FXR agonist M480 or THRβ agonist MGL-3196 (both dosed 10 mg/kg, qd, po).



To examine fibrosis in the absence of steatosis, a separate cohort of male C57BL/6 mice were orally dosed bi-weekly with 0.5ug/g CCl4 for 8 wks. Treatment with M480, MGL-3196 (each 10 mg/kg, qd, po) or TGF-βR1 inhibitor SB-525334 (30 mg/kg bid, po) was initiated after 3 wks of CCl4 exposure and continued for 5 weeks.

CONCLUSIONS

Development of NASH begins with the accumulation of hepatic lipids. Consequently, many drugs in clinical development for NASH are focused on reducing steatosis. While FXR and THRβ activation both decrease steatosis, FXR activation led to superior fibrosis improvement. FXR activation can decrease inflammation and fibrosis in models lacking hepatic lipid accumulation, suggesting FXR provides benefits in addition to and independent of decreasing steatosis.

RESULTS

Efficacy in Advanced Fibrosis DIO-NASH Model

Figure 1. FXR and THRβ activation reduce A) hepatic triglycerides and B) hepatic cholesterol in DIO-NASH mice with advanced fibrosis

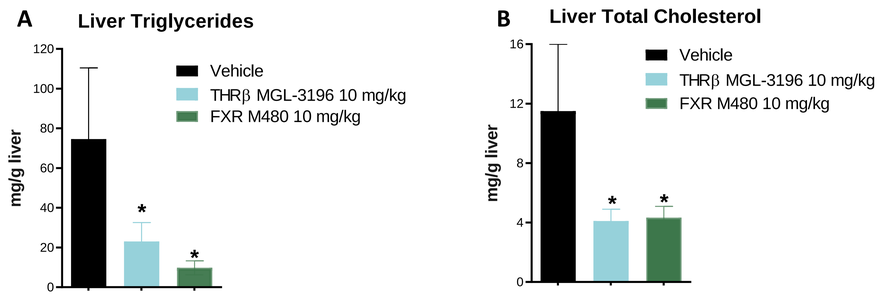


Figure 2. FXR activation shows better efficacy than THRβ in improving histological A) NAFLD activity score and B) fibrosis stage in DIO-NASH mice with advanced fibrosis

Pre, pre-biopsy at week -3 before treatment; Post, biopsy at week 8 post treatment. NAFLD activity score and fibrosis stage based on Kleiner 2005 Hepatology: Scores: 3 – bridging; 2 – perisinusoidal & portal/periportal

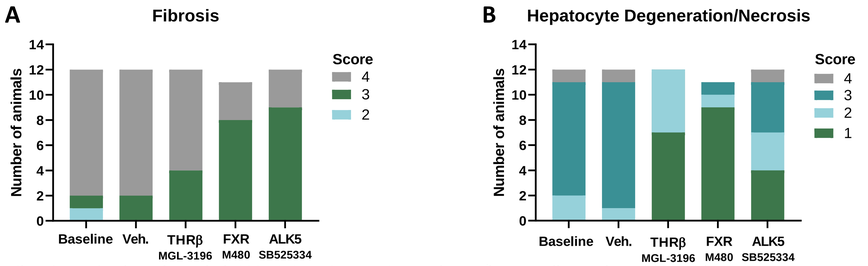
Table 1. Clinical chemistry and histopathology scores – FXR and THRβ activation similarly reduce steatosis, but FXR activation show superior efficacy in all other histological endpoints of NASH and fibrosis

	Terminal Plasma Clinical Chemistry and Histopathology Scores Mean (* p<0.05 One-Way ANOVA vs Vehicle)		
	Vehicle	THRβ MGL-3196	FXR M480
Triglycerides (mmol/L)	0.71	0.32*	0.42*
Cholesterol (mmol/L)	6.6	1.2*	3.1*
ALT (U/L)	153	219	139
AST (U/L)	199	329	242
Steatosis Score	2.8	1.1*	0.8*
Inflammation Score	2.0	2.6*	1.9
Ballooning Score #	0.2	0	0
NAFLD Activity Score	4.9	3.6*	2*
Col1a1 % fractional area	10.4	11.7	8.9
α-SMA % fractional area	3.0	4.1*	1.2*
Galectin-3 % factional area	3.5	4.6*	2.3*

ALT, alanine aminotransferase; AST, aspartate aminotransferase; Col1a1, collagen 1a1; α-SMA, alpha smooth muscle actin; # pre-dose ballooning scores averaged 0 for all treatment groups

Efficacy in CCl4 Fibrosis Model

Figure 3. Histological assessment of A) fibrosis and B) hepatocyte degradation & necrosis shows FXR activation exhibits better efficacy than THRβ activation or TGF-βR1 (ALK5) inhibition in CCl4 induced fibrosis



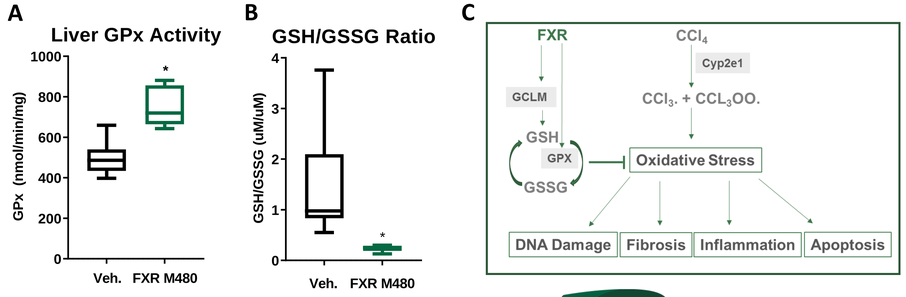
Fibrosis scoring based on Ishak 1995 J Hep. Scores: 4 – moderate/heavy collagen deposition with bridging; 3 – light/moderate collagen deposition with occasional bridging; 2 – centrilobular

Table 2. Liver histopathology and gene expression analysis – FXR activation decreases inflammation, reduces fibrotic gene expression and increases genes associated with glutathione metabolism

	Histopathology Scores & q-PCR Quantitation Mean (* p<0.05 One-Way ANOVA vs Vehicle)				
	Baseline	Vehicle	THRβ MGL-3196	FXR M480	ALK5 Inh. SB526334
Liver Inflammation Score	3.0	3.0	2.3*	1.8*	2.4*
Col 1a1 mRNA REL	0.6*	1.1	0.4*	0.1*	0.1*
Col 3a1 mRNA REL	0.6*	1.0	0.1*	0.1*	0.1*
Lgals3 mRNA REL	1.0	1.1	0.3*	0.2*	1.0
Cyp2e1 mRNA REL	0.9	1.1	1.3	2.5*	2.1
GPX1 mRNA REL	1.0	1.0	1.9*	2.3*	1.1
GCLM mRNA REL	1.0	1.0	0.9	1.8*	1.3

Col1a1, collagen 1a1; Col3a1, collagen 3a1; LGALS3, galectin 3; Cyp2e1, cytochrome P450 2E1; GPX1, glutathione peroxidase 1; GCLM, glutamate-cysteine ligase modifier subunit; REL - Relative Expression Level – all groups normalized to vehicle control

Figure 4. FXR activation leads to increased hepatic A) glutathione peroxidase activity and B) decreased GSH/GSSG ratio in CCl4 treated mice. C) Proposed FXR mechanism – increased glutathione metabolism likely decreases oxidative stress



DISCLOSURES

B.W., S.G., J.N., K.D., A.M., K.L., A.O., D.Z., J.Q., N.L., E.B., K.S. and N.S. are employees and equity holders in Metacrine, Inc.

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