Preclinical Animal Models for Nonalcoholic Steatohepatitis (NASH) and Their Pharmacological Validation

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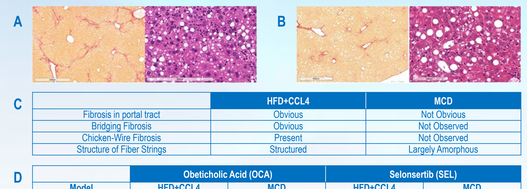
ABSTRACT

Background and aims: Preclinical animal models are essential to evaluating therapeutic agents for NASH (nonalcoholic steatohepatitis). Their clinical relevance underscores the validity of efficacy tests.

Method: We selected two animal models, MCD (induction with methionine and choline deficient diet) and HFD+CCL4 (sequential induction with high fat-diet followed by carbon tetrachloride) in mouse for histopathological analyses of hepatic steatosis, ballooning, inflammation and fibrosis, and efficacy tests with agents in clinical trials.

Results: Both models presented histopathological features that are consistent with the clinical definition of NAFLD activity score (NAS) and the hepatic fibrosis. However, periportal and perilobular bridging fibrosis (green and blue arrows in the figure below) was observed only in the HFD+CCL4 model (see Figure). The overall transcriptional changes (including inflammatory responses and lipid metabolism) in the livers of both HFD+CCL4 and MCD models displayed no correlation. The efficacy of obeticholic acid (OCA) was observed only in the HFD+CCL4 model, and that of selonsertib (SEL) only in the MCD model. The antifibrotic efficacy of OCA in the HFD+CCL4 model is exerted on perisinusoidal and portal tract fibrosis, and bridging fibrosis. In addition to OCA (targeting FXR) and SEL (ASK1), clinical agents targeting PPAR-alpha/delta, pan-PPAR, CCR2/CCR5, THR-beta, ACC1/2 and SSAO/VAP-1, and GLP1 were tested in the HFD+CCL4 model. Their efficacies were largely consistent with the respective clinical outcomes.

Conclusions: The HFD+CCL4 model is clinically relevant in histopathological findings. The results of pharmacological validation with clinical agents match the overall clinical outcomes.



	HFD+CCL4	MCD
Fibrosis in portal tract	Obvious	Not Obvious
Bridging Fibrosis	Obvious	Not Observed
Chicken-Wire Fibrosis	Present	Not Observed
Structure of Fiber Strings	Structured	Largely Amorphous

	Obeticholic Acid (OCA)		Selonsertib (SEL)	
Model	HFD+CCL4	MCD	HFD+CCL4	MCD
Steatosis	Improved	Improved	No effect	Improved
Inflammation	Improved	Exacerbated	Exacerbated	Improved
Ballooning	(Improved)	Exacerbated	Improved	No Effect
Fibrosis	Improved	No Effect	No Effect	Improved

Figure I-4. HFD+CLL4 and MCD models are mechanistically different. A & B. Liver histopathology of HFD+CLL4 (A) and MCD (B) models, stained with Sirius red (left) and HE (right). C. Comparison of liver fibrosis observed in both models. D. Pharmacological outcomes of OCA and SEL in both models.

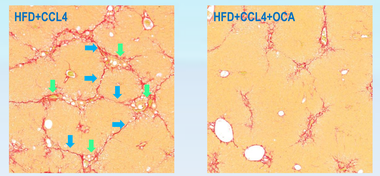


Figure III-1. Clinical relevance of the HFD+CCL4 model. The combination of HFD and CCL4 induces extra fibrosis in the perisinusoidal zone, long and thick fibers forming bridging fibrosis (blue arrows) and periportal fibrosis (green arrows) (left, and data not shown). These three types of fibrosis are the target of OCA (right, and data not shown). The bridging fibrosis is associated with steatosis in perisinusoidal zone (data not shown). These results indicate that the hepatic fibrosis in the HFD+CCL4 model is clinically relevant to that observed in NASH.

Compounds	Targets	Efficacy Test Results		Clinical Trial Status
		NAS	Fibrosis	
Obeticholic acid (INT-747)	FXR	Yes	Yes	P3, Completed
Selonsertib (GS4997)	ASK1	No	No	P3, Failed
Elafibranor (GFT-505)	PPAR-α/δ	No	Yes	P3, Failed
Cenicriviroc (TAK-652)	CCR2/CCR5	No	No	P3, Terminated
Resmetirom (MGL-3196)	THR-β	Yes	Yes	P3, Ongoing
VK2809	THR-β	Yes	Yes	P2b, Ongoing
Firsocostat (ND630)	ACC1/2	Yes	Yes	P2, Discontinued (due to increase blood TG)
Liraglutide	GLP1	Yes	No	P2, Completed
Tropifexor (LJN452)	FXR	Yes	Yes	P2, Ongoing
PXS 4728A	SSAO/VAP-1	No	No	P2, Discontinued
Lanifibranor (IVA337)	PPAR-α/δ/γ	Yes	Yes	P3, Ongoing
Aramchol	SCD-1	No	No	P3, Ongoing

Figure III-4. Summary of efficacy tests of NASH compounds in clinical trials. For most of these compounds, the results of efficacy tests in the HFD+CCL4 model are largely consistent with their respective clinical outcomes, suggesting that the model is clinically relevant.

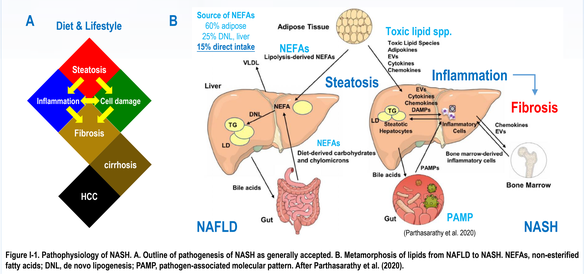
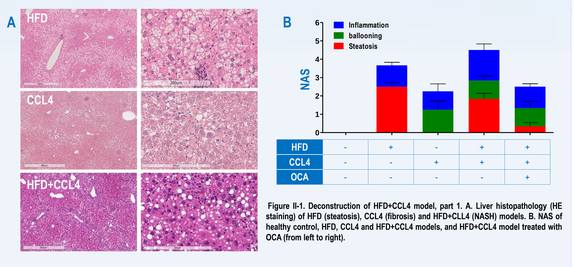


Figure I-1. Pathophysiology of NASH. A. Outline of pathogenesis of NASH as generally accepted. B. Metamorphosis of lipids from NAFLD to NASH. NEFAs, non-esterified fatty acids; DNL, de novo lipogenesis; PAMP, pathogen-associated molecular pattern. After Parthasarathy et al. (2020).



HFD	-	+	-	+	+
CCL4	-	-	+	+	+
OCA	-	-	-	-	+

Figure II-1. Deconstruction of HFD+CCL4 model, part 1. A. Liver histopathology (HE staining) of HFD (steatosis), CCL4 (fibrosis) and HFD+CCL4 (NASH) models. B. NAS of healthy control, HFD, CCL4 and HFD+CCL4 models, and HFD+CCL4 model treated with OCA (from left to right).

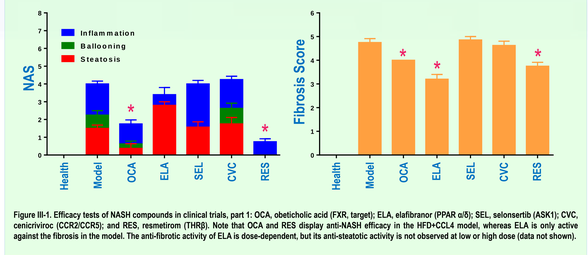


Figure III-1. Efficacy tests of NASH compounds in clinical trials, part 1: OCA, obeticholic acid (FXR, target); ELA, elafibranor (PPAR α/δ); SEL, selonsertib (ASK1); CVC, cenicriviroc (CCR2/CCR5); and RES, resmetirom (THRβ). Note that OCA and RES display anti-NASH efficacy in the HFD+CCL4 model, whereas ELA is only active against the fibrosis in the model. The anti-fibrotic activity of ELA is dose-dependent, but its anti-steatotic activity is not observed at low or high dose (data not shown).

Animal models for NASH and liber fibrosis provided by WuXi Biology

Diseases	Animal Models
NASH	HFD induction (NHP)
	HFD+CCL4 induction (mouse)
	MCD induction NASH-like (mouse, for hepatic inflammation)
	CDAHFD induction (mouse & rat)
Fibrosis	CCL4 induction (mouse & rat)
	TAA induction (Mouse & rat)
	Bile duct ligation (BDL) (mouse & rat)
Cirrhosis	CCL4 induction (rat)
	BDL (rat)
Primary Biliary Cholangitis (PBC)	BDL (mouse & rat)
	ANIT induction (mouse & rat)
Primary Sclerosing Cholangitis (PSC)	DDC induction (mouse)

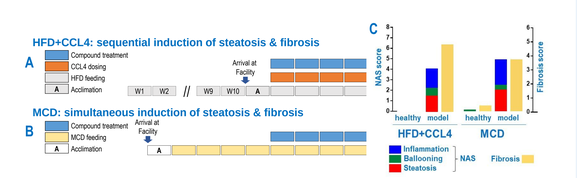
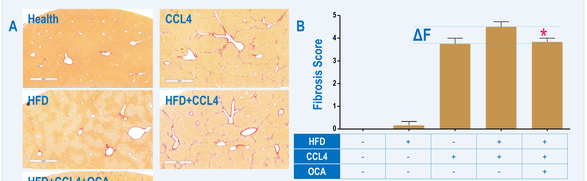


Figure I-2. Two mouse models for NASH. A. High-fat diet and CCL4 model of sequential induction (HFD+CCL4), animals are first induced for obesity by HFD (~10 weeks), and then for fibrosis by CCL4 (4 weeks). B. Methionine choline deficiency model of simultaneous induction (MCD). The depletion of methionine and chlorine in diet impairs export of triglyceride (TG) from hepatocytes and increases oxidative stress, which in turn result in steatosis, inflammation and fibrosis. C. Liver histopathology of HFD+CLL4 and MCD models. Liver slices are assessed for steatosis, ballooning and inflammation, the sum of which is known as NAFLD Activity Score (NAS), and fibrosis.



HFD	-	+	-	+	+
CCL4	-	-	+	+	+
OCA	-	-	-	-	+

Figure II-2. Deconstruction of HFD+CCL4 model, part 2. A. Liver histopathology of health, HFD, CCL4 and HFD+CCL4 models and HFD+CCL4 model treated with OCA (Sirius red staining). B. Fibrosis scores of five animal groups shown in A. Note that in the NASH model (i.e., HFD+CCL4), the combination of HFD and CCL4 induced extra fibrosis (ΔF) which was suppressed by OCA.

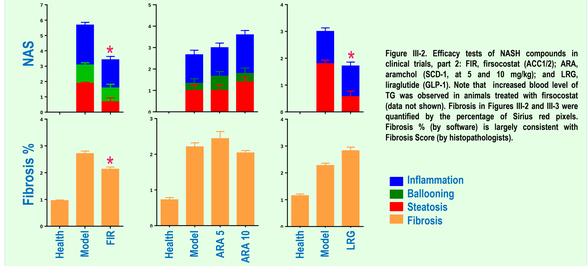


Figure III-2. Efficacy tests of NASH compounds in clinical trials, part 2: FIR, firsocostat (ACC1/2); ARA, aramchol (SCD-1, at 5 and 10 mg/kg); and LRG, liraglutide (GLP-1). Note that increased blood level of TG was observed in animals treated with firsocostat (data not shown). Fibrosis in Figures III-2 and III-3 were quantified by the percentage of Sirius red pixels. Fibrosis % (by software) is largely consistent with Fibrosis Score (by histopathologists).

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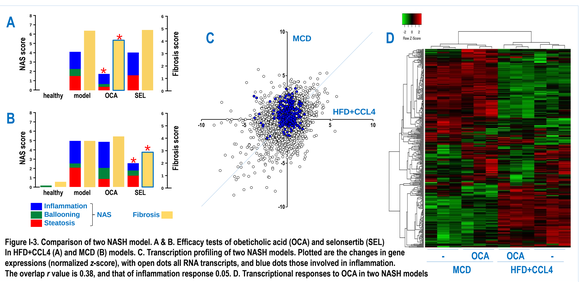

Figure I-3. Comparison of two NASH model. A & B. Efficacy tests of obeticholic acid (OCA) and selonsertib (SEL) In HFD+CCL4 (A) and MCD (B) models. C. Transcription profiling of two NASH models. Plotted are the changes in gene expressions (normalized z-score), with open dots all RNA transcripts, and blue dots those involved in inflammation. The overlap *r* value is 0.38, and that of inflammation response 0.05. D. Transcriptional responses to OCA in two NASH models

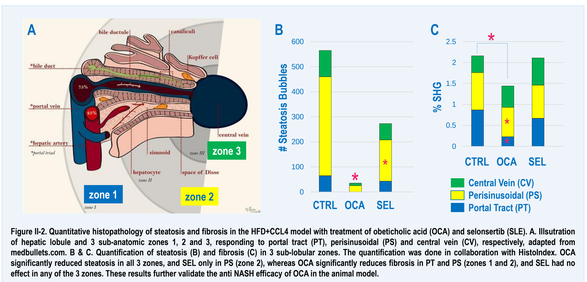

Figure II-2. Quantitative histopathology of steatosis and fibrosis in the HFD+CCL4 model with treatment of obeticholic acid (OCA) and selonsertib (SLE). A. Illustration of hepatic lobule and 3 sub-anatomic zones 1, 2 and 3, responding to portal tract (PT), perisinusoidal (PS) and central vein (CV), respectively, adapted from medbullets.com. B & C. Quantification of steatosis (B) and fibrosis (C) in 3 sub-lobular zones. The quantification was done in collaboration with HistoIndex. OCA significantly reduced steatosis in all 3 zones, and SEL only in PS (zone 2), whereas OCA significantly reduces fibrosis in PT and PS (zones 1 and 2), and SEL had no effect in any of the 3 zones. These results further validate the anti NASH efficacy of OCA in the animal model.

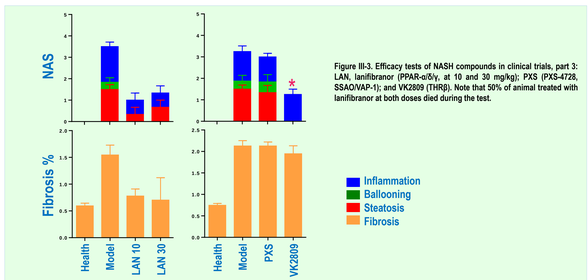

Figure III-3. Efficacy tests of NASH compounds in clinical trials, part 3: LAN, lanifibranor (PPAR-α/δ/γ, at 10 and 30 mg/kg); PXS (PXS-4728, SSAO/VAP-1); and VK2809 (THRβ). Note that 50% of animal treated with lanifibranor at both doses died during the test.

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