Cyclophilin inhibition with rencofilstat shifts the liver transcriptome and lipidome in preclinical models toward resolution of nonalcoholic steatohepatitis

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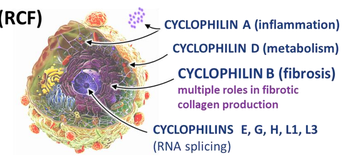
INTRODUCTION

CYCLOPHILINS: multi-isoform isomerase enzymes that influence the function of numerous, diverse proteins through regulation of proline-bond *cis-trans* conformation

RENCOFILSTAT (formerly CRV431): a cyclophilin inhibitor currently in Phase 2 clinical trials for NASH and HCC. Key activities of rencofilstat include anti-fibrotic, anti-inflammatory, and metabolic regulation.

RENCOFILSTAT (RCF)
inhibits multiple cyclophilin enzymes that contribute to NASH pathogenesis



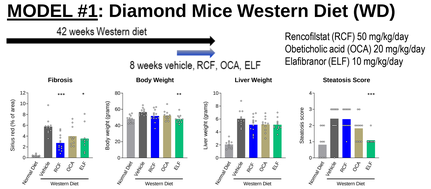
AIMS

Characterize rencofilstat's effects on the liver transcriptome and lipidome in three liver disease models.

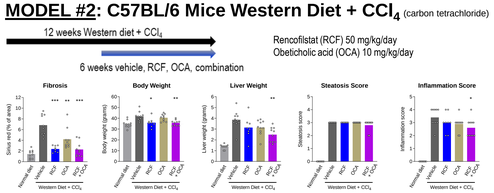
METHODS

TRANSCRIPTOMIC and LIPIDOMIC ANALYSES IN 3 MODELS:

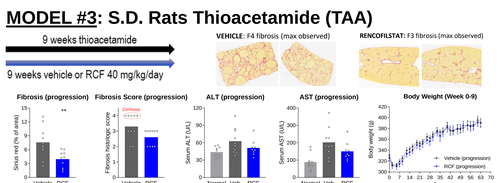
MODEL #1: Diamond Mice Western Diet (WD)



MODEL #2: C57BL/6 Mice Western Diet + CCl_4 (carbon tetrachloride)



MODEL #3: S.D. Rats Thioacetamide (TAA)



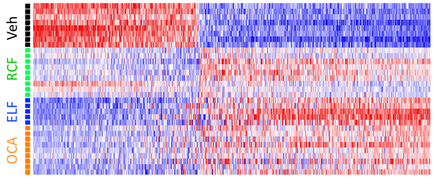
LIVER TRANSCRIPTOMICS: Differentially Expressed Genes (DEGs)

DIAMOND MICE – Western Diet (WD)

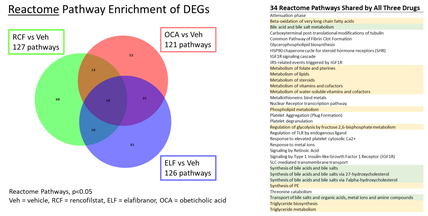
Rencofilstat DEGs significantly overlapped with elafibranor and obeticholic acid DEGs

% OVERLAP OF DEGs (DEGs vs Vehicle)

	Rencofilstat	Elafibranor	OCA
Rencofilstat (1576 DEGs)		**74%** (1169/1576)	**81%** (1270/1576)
Elafibranor (4577 DEGs)	**26%** (1169/4577)		**55%** (2529/4577)
OCA (4216 DEGs)	**30%** (1270/4216)	**60%** (2529/4216)	



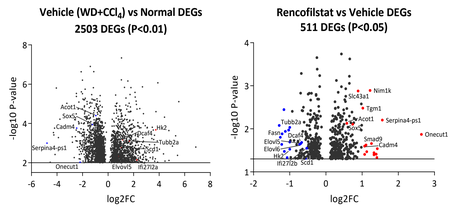
Predominance of lipid and bile pathways in common among RCF, ELF, and OCA DEGs



C57BL/6 MICE – Western Diet (WD) + CCl_4

Rencofilstat opposed many significant DEGs associated with Western diet + CCl_4

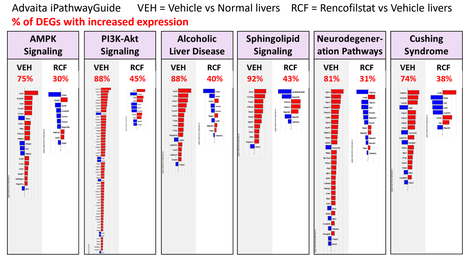
e.g. Fasn, Scd1, Acot1, SerpinA4-ps1, Oncecut1, HK2, Elvovl5, Sox5



KEGG pathways active in diseased livers were commonly opposed by rencofilstat (RCF)

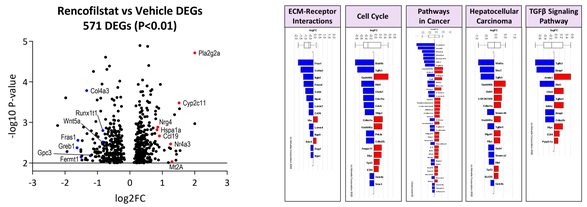
Advaita iPathwayGuide VEH = Vehicle vs Normal livers RCF = Rencofilstat vs Vehicle livers

% of DEGs with increased expression

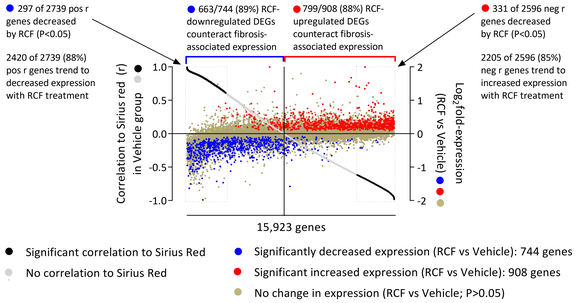


RATS – Thioacetamide (TAA)

Rencofilstat DEGs highly associated with attenuation of disease pathways

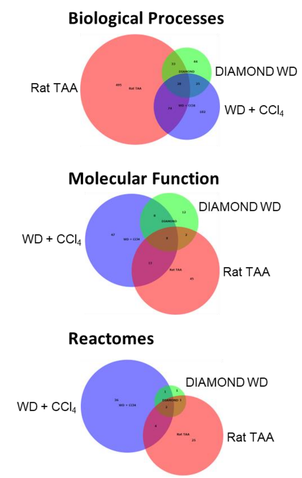


Rencofilstat preferentially reversed the transcriptional changes of fibrosis-correlated genes (Vehicle group Sirius red)



Gene Ontologies

Rencofilstat consistently exerted anti-fibrotic and other therapeutic effects, but the types of processes and functions altered by rencofilstat *based on transcriptional changes* differed significantly among disease models.



RCF DEG Summary

Model	Vehicle n	RCF n	P-value	# DEGs
HUMAN PCLS (stim)	3	3	P<0.05	250
HUMAN PCLS (nonstim)	3	3	P<0.05	408
DIAMOND Mice + WD	8	9	Padj<0.05	1650
C57BL/6 Mice + WD + CCl_4	5	4	P<0.05	511
STAM Mice + WD	9	8	Padj<0.05	1754
RAT TAA	10	10	P<0.01	690

5,263 genes altered by rencofilstat

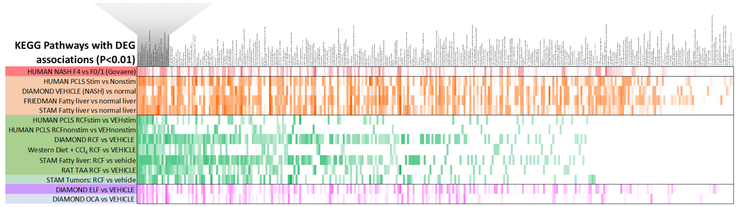
80% of rencofilstat's DEGs are present in only one model, indicating a major role of disease-specific processes in transcriptional responses to rencofilstat

KEGG Pathways: Human F4 NASH vs Disease Models

KEGG Pathway	HUMAN NASH F4 vs NAFLD F0/1 (Govaere 2020)	NASH MODELS VEHICLE Average	NASH MODELS RENCOFILSTAT Average	NASH MODELS ELF + OCA Average
Metabolic pathways	-5.11	-5.11	-5.11	-5.11
Pathways in cancer	-5.56	-5.58	-4.65	-5.11
Human papillomavirus infection	-5.51	-5.42	-3.94	-4.71
PI3K-Akt signaling pathway	-4.89	-6.11	-3.93	-5.25
Human cytomegalovirus infection	-5.73	-5.18	-3.70	
Alcoholic liver disease	-4.20	-4.01	-4.19	-4.25
Pathways of neurodegeneration - multiple diseases	-3.75	-4.25	-3.98	-3.52
Alzheimer disease		-4.38	-3.80	-3.59
Rap1 signaling pathway	-4.90	-5.45	-3.77	-4.94
Herpes simplex virus 1 infection		-4.00	-3.72	-2.00
FoxO signaling pathway	-2.37	-4.26	-3.65	-3.31
Coronavirus disease - COVID-19	-2.03	-5.99	-3.46	-3.17
Human T-cell leukemia virus 1 infection	-3.30	-5.29	-3.28	-2.33
Cushing syndrome	-6.01	-4.29	-2.95	-2.76
Kaposi sarcoma-associated herpesvirus infection	-5.79	-5.02	-2.88	
MAPK signaling pathway	-5.01	-5.22	-5.40	-6.74
Protein processing in endoplasmic reticulum		-2.90	-4.90	-4.59

The top KEGG pathways associated with rencofilstat transcriptional effects in preclinical models overlap significantly with human F4 NASH

(data from Govaere et al 2020; log10 P-values describing DEG association with KEGG pathways)

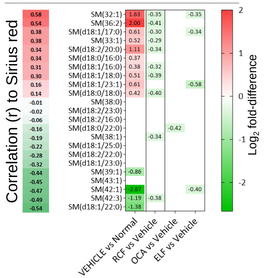


LIVER LIPIDOMICS

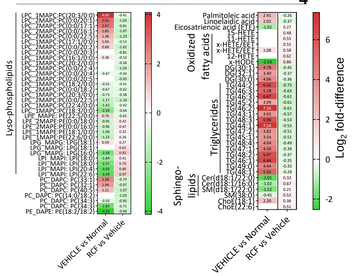
Rencofilstat exerted normalizing effects on several lipid classes in diseased livers, including lipids with known toxicities:

- *sphingolipids, lysophospholipids, triglycerides (especially shorter chain), oxidized fatty acids*

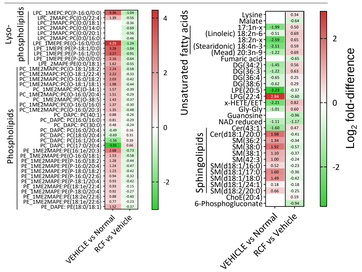
DIAMOND MICE - WD



C57BL/6 MICE- WD + CCl_4



RATS - Thioacetamide



CONCLUSIONS

- Rencofilstat exerted liver antifibrotic effects in all disease models
- Rencofilstat consistently attenuated disease-associated changes in gene expression and lipids, but the specific alterations were distinctive to each model
- Rencofilstat affected KEGG pathways that also occur in advanced human NASH