Rencofilstat Multiomics Indicate Clinically Important Mechanisms in NAFLD-NASH.

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Background: Progression of liver fibrosis in Non-Alcoholic Steatohepatitis (NASH) has been directly linked to increased mortality and morbidity. Rencofilstat (RCF, formerly CRV431), is a non-immunosuppressive cyclophilin (Cyp) inhibitor that has demonstrated anti-fibrotic effects in numerous pre-clinical fibrosis studies, and specifically in NASH subjects. A multi-omics analysis of transcriptomics and lipidomics was performed to further elucidate the action of RCF in subjects with biomarker defined F2/F3 NASH and explore biomarkers markers related to clinical responsive.

Methods: RNA sequencing data with serum lipid analysis was obtained from 27 patients on active RCF treatment, with biomarker confirmed F2/F3 NASH participating in a 28-day, Phase 2a trial (NCT04480710). A total of 43 subjects were administered RCF 75 mg, 225 mg, or placebo orally once daily for 28 days. RNA was stabilized and isolated from whole blood on Day 1 and Day 28. RNA sequencing transcripts were evaluated using FastQC, with quantification in Salmon v1.4.0. Differential expression analysis (DEA) was performed using edgeR and Advaita Bioinformatics iPathway. Serum lipid levels were quantitated by Owl metabolomics. Multi-omic analysis was performed using a projection to latent structures (PLS) method as implemented in the Bioconductor package, mixOmics. Lipid/transcriptomics were evaluated in terms of clinical outcome trait measures including ALT, AST, ProC3, C3M, C6M, PLT, and FIB-4. Final lipid-gene networks were identified to determine exposure to RCF and ProC3 reduction. ProC3 response was taken as any reduction from baseline in ProC3 by at least 2 ng/mL. The final gene network was analyzed using weighted key driver analysis as implemented in Bioconductor package, Mergeomics.

Results: Differential gene expression demonstrated significant effects of RCF on key factors in the KEGG NAFLD/NASH pathway. These genes could also be functionally linked to individual cyclophilins.

Kegg Gene/Protein	Effect of RCF	Cyclophilin(s)	Function(s)
IL6	IL6R Downregulation	A	Anti-inflammatory
Akt	AKT2 Upregulation	A	Insulin secretion/resistance, hepatocyte growth factor signaling
AMPK	PRKAG1 Upregulation	D	Hepatic glucose production/lipogenesis
JNK1/2	MAPK8 Upregulation	A/B	PPAR related activity
CASP3	CASP3 Downregulation	A/D	Block apoptosis & hepatocyte injury
CXI/II	NDUFA5, NDUC1, SDHA Downregulation	B/D	Mitochondrial Fatty AcylCoA β-oxidation

ProC3 response could be predicted by 25 key genes (**AUROC= 0.97**). Key driver analysis of the entire gene network identified PCOLCE, MAPK7, MYH9, & GCLC as top key drivers with JAK1 as a key driver.
Conclusions: Multi-omic analyses has linked Rencofilstat activity directly to classical NAFLD/NASH pathways and identified a panel of genes and lipids that can accurately predict ProC3 response. The top key driver, Procollagen C-endopeptidase Enhancer (PCOLCE) encodes for Procollagen C-Proteinase Enhancer 1 (PCPE1) which is a fibrosis marker and potential therapeutic target. Rencofilstat demonstrates pleotropic antifibrotic activity which may provide synergy with other NASH compounds in development.

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