Rencofilstat Multiomics Data 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 recently in NASH subjects. A multiomics analysis of transcriptomics and lipidomics was performed in subjects with biomarker-defined F2/F3 NASH to further elucidate the action of RCF and to explore biomarkers related to clinical response.

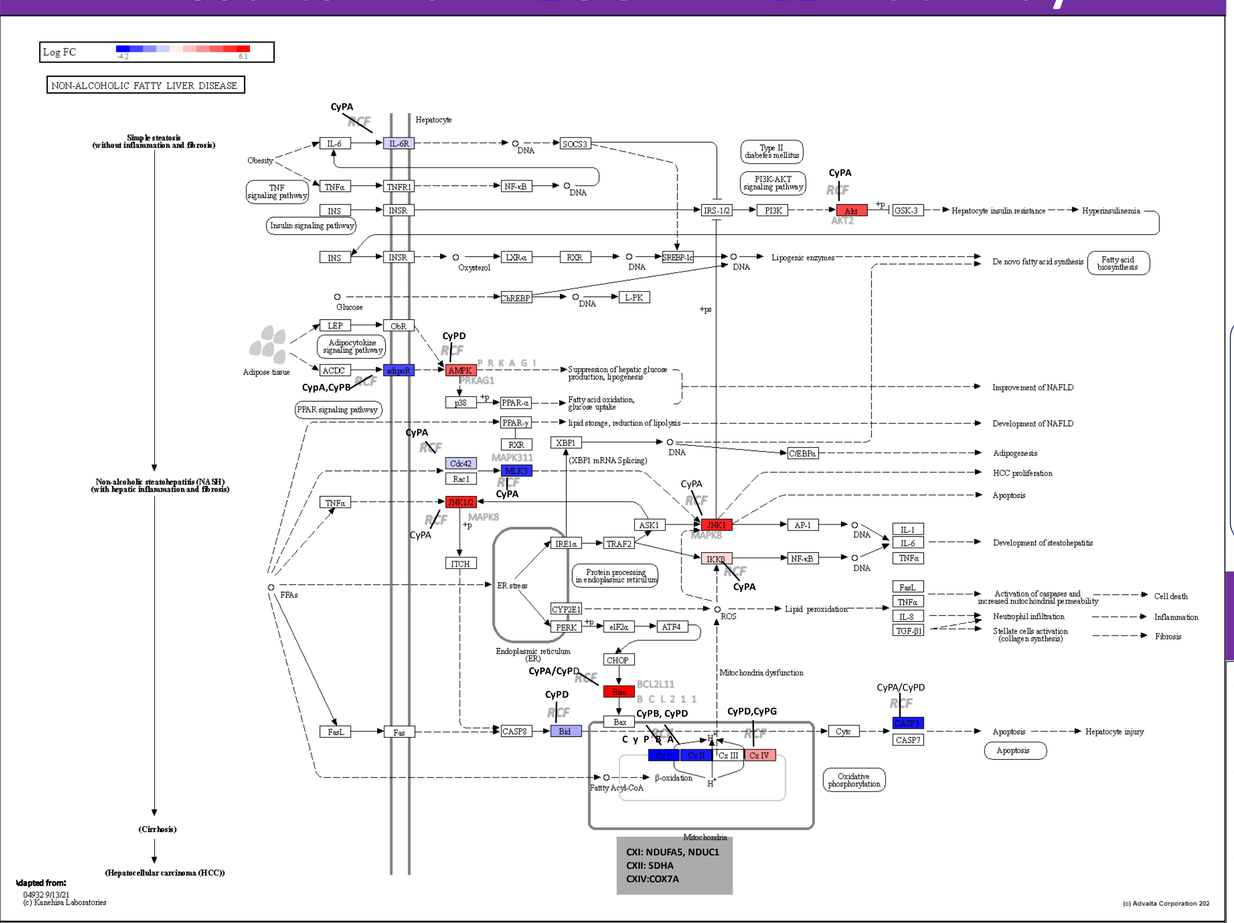
Objectives

- The primary objectives of this Phase 2a Study were to assess the safety, tolerability, and pharmacokinetics (PK) of Rencofilstat in subjects with presumed NASH fibrosis stage 2 or 3.
- Exploratory endpoints evaluated NASH biomarkers including whole blood transcriptomics and serum lipidomics.

Methods

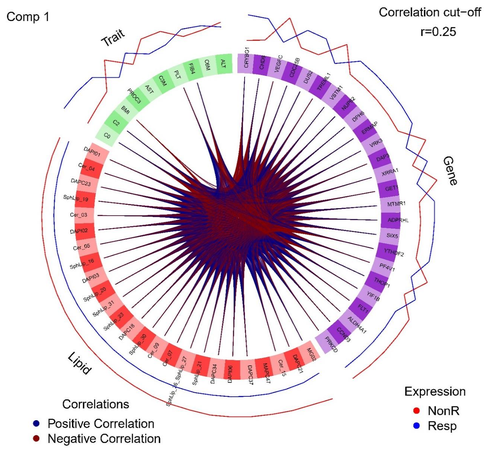
RNA sequencing data with serum lipid analysis was obtained from 27 subjects on active RCF treatment, with biomarker confirmed F2/F3 NASH participating in a 28-day, Phase 2a trial (NCT04480710). A total of 43 subjects were administered RCF 75 mg, 225 mg, or placebo orally once daily for 28 days. RNA was 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. Multiomic 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 defined 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 the Bioconductor package, Mergeomics.

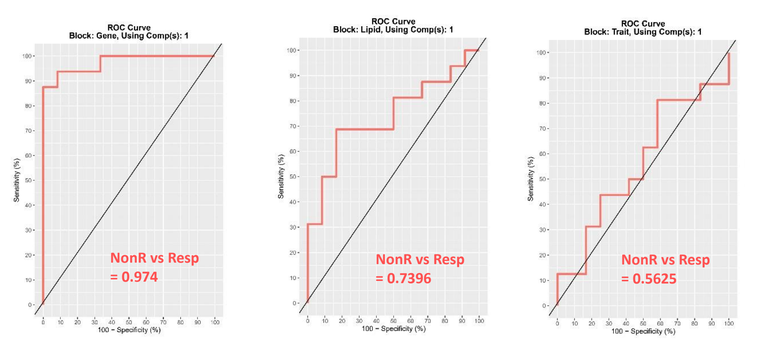
Results: RCF-KEGG NAFLD Pathway



- The role of cyclophilin-inhibition for RCF is shown in the current KEGG NAFLD Pathways.
- Differentially expressed genes are based on mRNA measured in whole blood, which may differ from tissue sampled mRNA.
- mRNA may not adequately predict proteomics, therefore this has been added to an ongoing Phase 2B study.

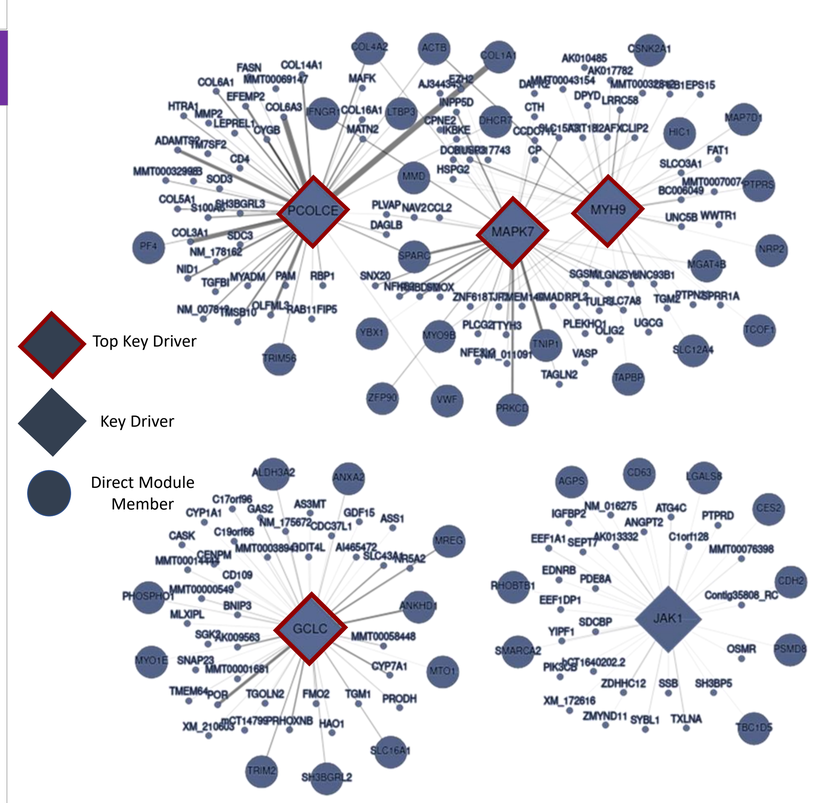
Multiomics: Pro-C3 Responder Group





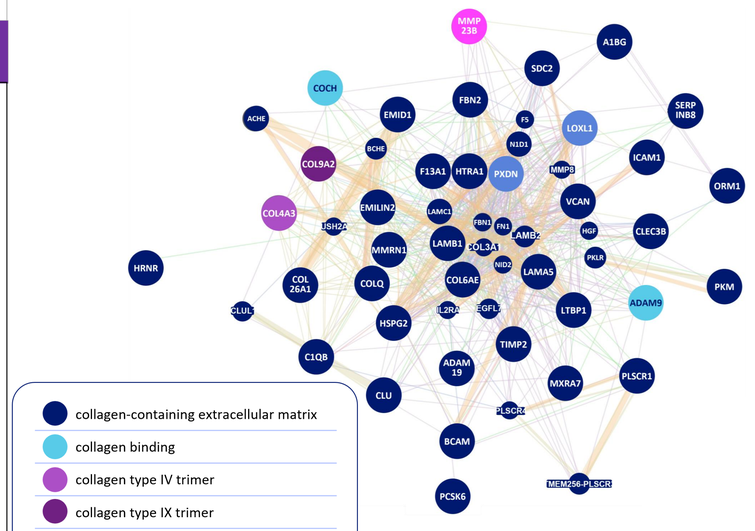
- 25 genes out of 1733 statistically significant genes provide good prediction for ProC3 response.
- 25 lipids demonstrate reduction in ceramides, free fatty acids, and triglycerides.
- Changes in clinical labs and Fib-4 were not good predictors of ProC3 response.

ProC3 Responder Network: Weighted Key Driver Analysis



- Procollagen C-endopeptidase Enhancer (PCOLCE) is the gene name for the protein Procollagen C-Proteinase Enhancer 1 (PCPE1) which has been identified as a potential biomarker and/or therapeutic target for fibrosis including liver fibrosis.
- PCPE1 regulates C-terminal procollagen processing and collagen fibril assembly.
- MYH9 acts via TGF-β1 on fibroblast-myofibroblast differentiation in lung fibrosis models.
- GCLC is a negative regulatory factor in HCV-related liver fibrosis.
- MAPK7 is part of the MAPK signaling pathway and has been shown to be modulated by CyPA and CyPD and is involved in NASH pathophysiology.
- JAK1 has been shown to possess both anti-inflammatory and antifibrotic effects in liver and lung fibrotic disease.

RCF - Collagen Regulatory Network



Non-Network Collagen Catabolism Genes	Proposed Mechanism
LARP6 (La Ribonucleoprotein 6)	Myofibroblast Inactivation
CTSB (Cathepsin B)	Collagen Catabolic Process
CTSK (Cathepsin K)	Collagen Catabolic Process
CTSL (Cathepsin L)	Collagen Catabolic Process
LTBP1 (Latent TGF-β-Binding Protein 1)	Coordinates fibrillin-1/2 into ECM

Conclusions

- Phase 2a transcriptomics suggest that RCF cyclophilin-inhibition is involved at multiple sites in the NASH/NAFLD pathophysiological pathway.
- Treatment with RCF for 28 days results in a reduction in ceramides, free fatty acids, and triglycerides suggestive of a therapeutic benefit.
- Multiomic analyses suggest that ProC3 RCF-responders can be identified based on 25 key genes
- RCF is involved in regulation of collagen synthesis and catabolism which is currently being studied for clinical relevance in two larger and longer Phase 2 clinical trials.