Integrated transcriptomics of Rencofilstat treatment in a Phase 2a NASH trial confirms anti-fibrotic effect of pan-cyclophilin inhibition and identifies Rencofilstat-specific biomarkers.

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INTRODUCTION

The progression of liver fibrosis in Non-Alcoholic Steatohepatitis (NASH) has been directly linked to increased mortality and morbidity. To date, numerous drug candidates have failed to show a significant benefit in fibrotic endpoints for NASH subjects enrolled in clinical trials. Rencofilstat (RCF), a non-immunosuppressive pan-cyclophilin inhibitor has demonstrated pleotropic effects well-suited for the treatment of NASH and fibrosis.

AIM

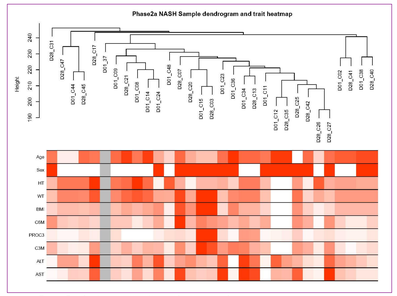
To use transcriptomics to elucidate the effects of RCF in patients with biomarker identified F2/F3 NASH subjects.

- Identify Gene – Clinical Trait Modules
- Identify Key Driver Genes in each Module
- Evaluate if RCF antifibrotic effect is linked to collagen synthesis and catabolism
- Develop a biomarker panel for RCF responsivity

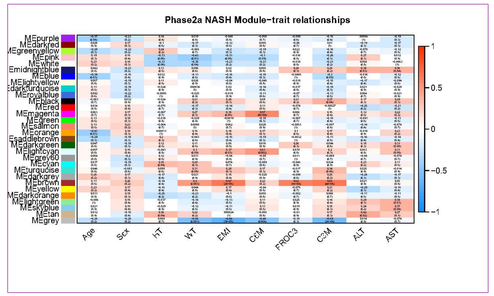
METHOD

- RNA sequencing data was obtained from a 28-day Phase 2a trial of RCF in F2/F3 NASH subjects (NCT04480710)
- Complete data was obtained from N=31 subjects on 75 mg or 225 mg RCF active treatment .
- 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 in R v4.1.1.
- Co-expression networks were constructed using weighted gene correlation network analysis (WGCNA).
- DEA was combined with WGCNA to enhance the discriminating ability of highly related genes as potential biomarkers.
- Weighted Key driver analysis (wKDA) was performed on trait modules using Mergeomics with network plotting and community detection in Cytoscape 3.9.1.
- Functional enrichment was performed using cosine similarity implemented via GeneWalk.

RESULTS

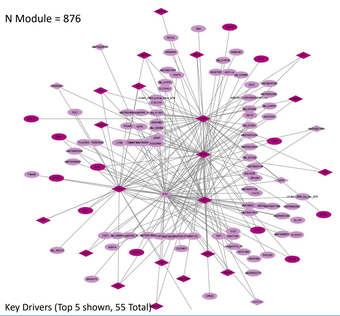


Grey: Subject excluded from final analysis



wKDA ALT Gene Module (r=0.59 p =8.9e-136)

N Module = 876

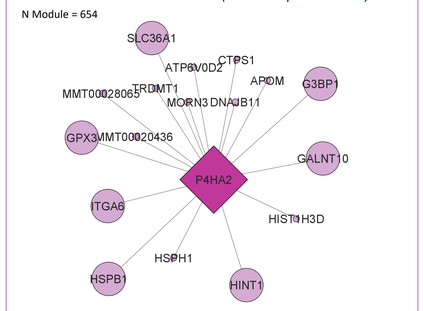


Key Drivers (Top 5 shown, 55 Total)

NODE	Name	FDR	N.neigh	N.obsrv	N.expct
IFI44	Interferon Induced Protein 44	4.12E-35	43	20	1.2
RTP4	Receptor Transporter Protein 4	1.48E-24	46	17	1.2
ISG15	Interferon-stimulated Gene 15	6.73E-22	27	16	0.73
IFIT1	Interferon Induced Protein With	1.97E-17	39	15	1.1
IFIT2	Tetratricopeptide Repeats 1 & 2	5.87E-17	52	15	1.4

wKDA ProC3 Gene Module (r=0.78 p < 1e-200)

N Module = 654



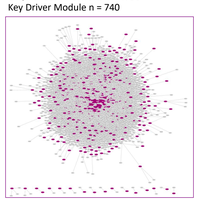
Key Drivers

NODE	Name	FDR	N.neigh	N.obsrv	N.expct
P4HA2	Prolyl 4-Hydroxylase Subunit Alpha 2	0.00243	18	8	0.4

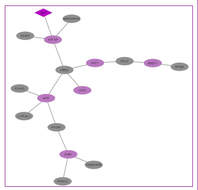
C6M - Gene Module (r=0.72 p <1e-200)

Community Detection: Fast Greedy

Original C6M Network n = 4750
Key Driver Module n = 740



Community: SP1

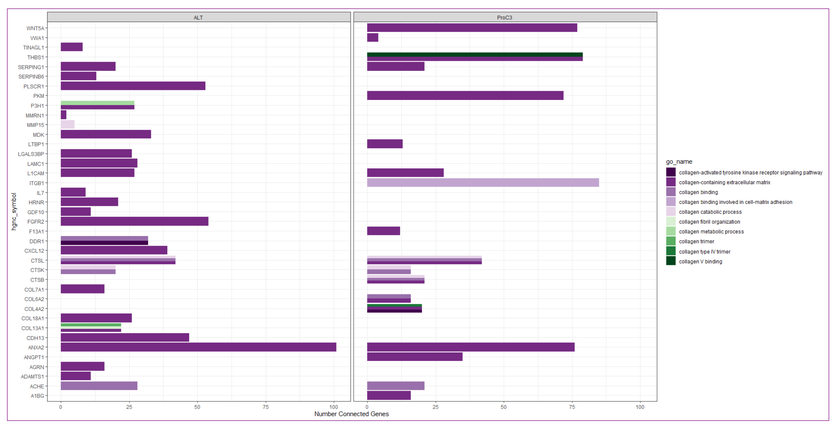


Key Drivers

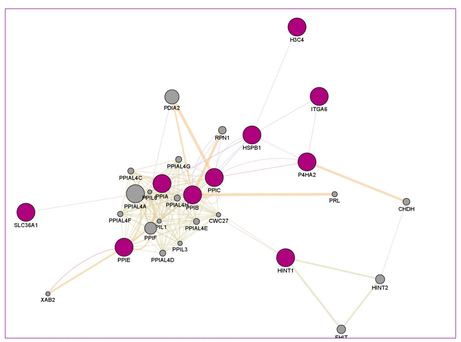
NODE	Name	FDR	N.neigh	N.obsrv	N.expct
KLF14	Kruppel Like Factor 14	0.004533	1593	170	118.9
SP1	Sp1 Transcription Factor	0.04244	5040	432	376.0
SP4	Sp2 Transcription Factor	0.072973	3110	283	232.0

Where: FDR = False Discovery Rate, N.Neigh=Number of Neighbors, N.Obsrv = Number Observed, N.expct = Number Expected

Genewalk: ALT & ProC3 Enrichment Collagen Gene Ontology Domains



ProC3 Key Driver Network Interactions with Cyclophilins



PPIA = Peptidylprolyl Isomerase A (Cyclophilin A)
PPIB = Peptidylprolyl Isomerase A (Cyclophilin B)
PPIC = Peptidylprolyl Isomerase C (Cyclophilin C)
PPIE = Peptidylprolyl Isomerase E (Cyclophilin E)
P4HA2 = Prolyl 4-Hydroxylase Subunit Alpha 2 (Collagen Prolyl 4-Hydroxylase Alpha II)

CONCLUSIONS

- The ALT-module demonstrated great heterogeneity in function including anti-inflammatory and anti-viral properties in addition to collagen regulation consistent with cyclophilin pharmacology.
- The C6M module had great heterogeneity in scope with only three key driver genes identified modulated by RCF treatment.
- The ProC3 module demonstrated the greatest specificity involving regulation of collagen synthesis.
- ProC3 Key Driver network directly interacts with cyclophilins.
- Treatment of these NASH subjects with RCF modulates a large collagen regulatory network including synthesis and catabolism.
- Key driver genes and their regulatory networks can be utilized as biomarkers for RCF pharmacodynamics.
- Gene networks identified in this study support further development in the treatment of NASH and hepatocellular carcinoma.

ACKNOWLEDGEMENTS

Dedicated to the patients that participated in trial NCT04480710.

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