Rencofilstat Exerts a Dominant Role in Synergistic Anti-PD1-Combination Effects in a Fatty Liver Model of Hepatocellular Carcinoma

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AACR ANNUAL MEETING 2023 Orlando

HEPION PHARMACEUTICALS

4125

INTRODUCTION

RENCOFILSTAT (RCF; cyclophilin inhibitor)

- Phase 2 clinical drug candidate for hepatocellular carcinoma (HCC) and nonalcoholic steatohepatitis (NASH)
- inhibits selected isoforms of cyclophilin isomerases

CYCLOPHILINS

- proline-directed isomerases that alter the structure, function, and molecular interactions of many proteins
- immunomodulatory, antifibrotic, cytostatic, and other anti-cancer activities

AIMS

- examine rencofilstat's anti-HCC effects alone and in combination with anti-PD1 IgG in a murine, syngeneic, orthotopic transplant model on the background of nonfatty versus fatty livers

METHODS

Hep53.4 Orthotopic HCC Transplant Model

Optional 3 months lead-up Western diet

Surgically implant murine Hep53.4 HCC cells into livers

Days 14-28 vehicle, rencofilstat, anti-PD1, or combination

Day 28 Sacrifice, measure tumors

Drug Treatments, Starting 14 Days Post HCC Implantation

- Rencofilstat – daily oral gavage 80 mg/kg
- Anti-PD1 IgG – 200 µg 2x/week intraperitoneal
- Combination rencofilstat + anti-PD1 IgG

Survival Analysis

- Orthotopic model on fatty liver background
- Treatments from Day 14 until termination

Immunohistochemical detection of tissue-infiltrating immune cells in tumor and nontumor tissue

RNA sequencing (bulk) of tumor and nontumor tissue

CONTACT INFORMATION

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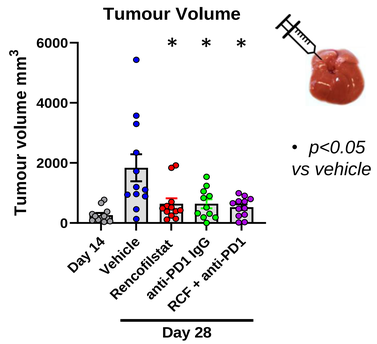
RESULTS

Orthotopic Tumors in NORMAL Livers

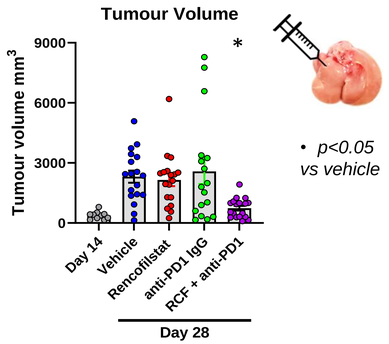
Monotherapy, anti-tumor effects from both rencofilstat and anti-PD1 IgG (~ 80% ↓)



• *p<0.05 vs vehicle*

Orthotopic Tumors in FATTY Livers

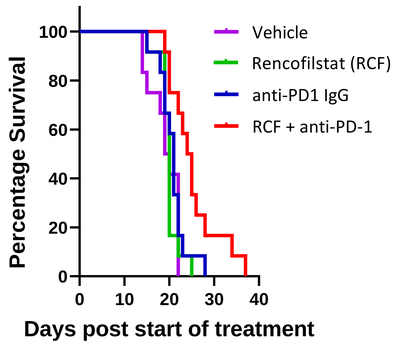
Tumor size decreased only with combination rencofilstat plus anti-PD1 IgG treatment (84% ↓)



• *p<0.05 vs vehicle*

Survival Analysis in FATTY Livers

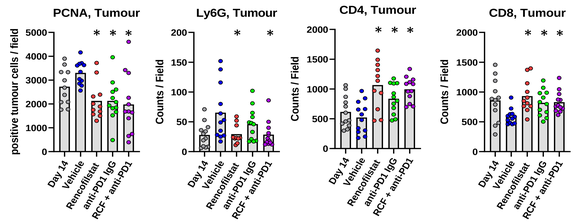
Rencofilstat plus anti-PD1 IgG extended survival by 26%



Tumor-Infiltrating Immune Cells in NORMAL Livers

Rencofilstat modulated CD4, CD8, *and* neutrophil infiltration

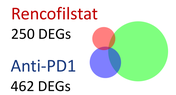
	PCNA	Neutrophils	CD4	CD8
Vehicle	-	-	-	-
Rencofilstat (RCF)	↓	↓	↑	↑
Anti-PD1	↓	-	↑	↑
RCF + anti-PD1	↓	↓	↑	↑



Tumor Differentially Expressed Genes (DEGs) vs Vehicle Group - RNAseq

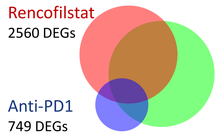
Rencofilstat exerted a much more significant effect on tumor gene expression in fatty livers

Tumors in NORMAL LIVERS



Tumors in NORMAL LIVERS	Overlap with Combination Group	Overlap % of RCF DEGs	Overlap% of anti-PD1 DEGs	Overlap % of Combination Group DEGs
Rencofilstat (250 DEGs)	33 DEGs	13%		**2%**
Anti-PD1 IgG (462 DEGs)	125 DEGs		27%	**7%**

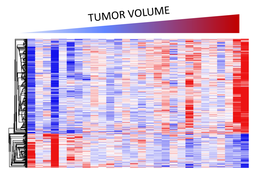
Tumors in FATTY LIVERS



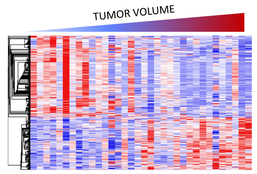
Tumors in FATTY LIVERS	Overlap with Combination Group	Overlap % of RCF DEGs	Overlap% of anti-PD1 DEGs	Overlap % of Combination Group DEGs
Rencofilstat (2560 DEGs)	1477 DEGs	58%		**49%**
Anti-PD1 IgG (749 DEGs)	383 DEGs		51%	**13%**

Gene Expression Correlated to Tumor Volume

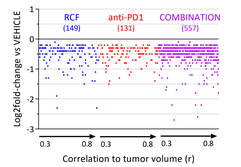
Tumors in NORMAL LIVERS

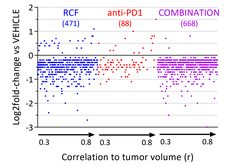


Tumors in FATTY LIVERS

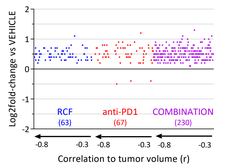


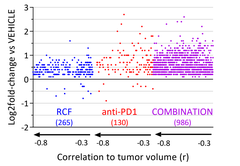
POSITIVELY CORRELATED GENES





NEGATIVELY CORRELATED GENES





The majority of rencofilstat and anti-PD1's effects were consistent with reduction of tumor volume.

In *fatty liver* tumors, rencofilstat shifted over **3-times** more tumor volume-correlated genes than anti-PD1.

Tumors in NORMAL Livers: DEG Pathway Mapping

KEGG PATHWAY	RCF	Anti-PD1	COMBO
Axon guidance		-5.68	
Protein processing in endoplasmic reticulum	-5.28		
Cytokine-cytokine receptor interaction		-5.20	
Chemical carcinogenesis - reactive oxygen species			-5.14
Metabolic pathways	-5.11		-5.11
Aminoacyl-tRNA biosynthesis			-5.11
Proteasome		-5.11	
Osteoclast differentiation			-4.95
Chemokine signaling pathway		-4.74	
Huntington disease			-4.61
Peroxisome			-4.58
Prion disease	-3.09		-5.53
Diabetic cardiomyopathy			-4.25
Retrograde endocannabinoid signaling			-4.23
Viral protein interaction with cytokine and cytokine receptor		-4.20	
Neutrophil extracellular trap formation			-3.87
Oxidative phosphorylation			-3.83
Legionellosis	-3.78		
Chagas disease		-3.71	
Pathways of neurodegeneration - multiple diseases	-3.28		-4.00
Cysteine and methionine metabolism	-3.47		
Tryptophan metabolism			-3.30
Thermogenesis			-3.30
Rheumatoid arthritis		-4.16	-2.02

Tumors in FATTY LIVERS: DEG Pathway Mapping (#1)

KEGG PATHWAY	RCF	Anti-PD1	COMBO
Natural killer cell mediated cytotoxicity	-7.95	-5.10	-7.66
Cytokine-cytokine receptor interaction	-6.24	-5.94	-6.00
Th17 cell differentiation	-6.25	-5.80	-5.13
Chemokine signaling pathway	-6.36	-3.34	-6.86
Th1 and Th2 cell differentiation	-5.42	-4.84	-5.32
Hematopoietic cell lineage	-5.11	-5.11	-5.11
Human T-cell leukemia virus 1 infection	-5.06	-4.79	-4.87
T cell receptor signaling pathway	-4.86	-4.99	-4.68
Coronavirus disease - COVID-19	-5.43	-3.95	-4.95
Cell adhesion molecules	-4.33	-4.38	-5.11
PD-L1 expression and PD-1 checkpoint pathway in cancer	-4.86	-4.94	-3.65
B cell receptor signaling pathway	-4.44	-3.42	-4.98
JAK-STAT signaling pathway	-4.38	-3.49	-4.75
Systemic lupus erythematosus	-4.77	-2.35	-4.89
Primary immunodeficiency	-2.58	-5.11	-4.19
Yersinia infection	-5.24	-3.66	-2.55
NF-kappa B signaling pathway	-2.51	-4.32	-4.47
Leukocyte transendothelial migration	-3.34	-2.08	-5.25
Ras signaling pathway	-4.88	-2.28	-3.10
Ribosome	-4.83	-2.21	-3.14
Viral protein interaction with cytokine/receptor	-2.82	-4.49	-2.85
Chagas disease	-2.31	-5.09	-2.35
Autoimmune thyroid disease	-2.64	-2.29	-4.69
PI3K-Akt signaling pathway	-3.80	-2.21	-2.29

Tumors in FATTY LIVERS: DEG Pathway Mapping (#2)

KEGG PATHWAY	RCF	Anti-PD1	COMBO
Fc epsilon RI signaling pathway	-5.83		-4.40
Regulation of actin cytoskeleton	-5.31	-2.29	
Antigen processing and presentation	-5.03		-6.41
Rap1 signaling pathway	-4.85		-3.83
Viral myocarditis	-4.09		-5.74
Fc gamma R-mediated phagocytosis	-4.08		-3.33
Vascular smooth muscle contraction	-3.96		-2.86
Calcium signaling pathway	-3.64		-4.21
Kaposi sarcoma-associated herpesvirus infection	-3.61		-3.04
Platelet activation	-3.56		-2.14
Diabetic cardiomyopathy	-3.55		-3.50
Epstein-Barr virus infection	-3.48		-4.53
Allograft rejection	-3.33		-5.55
Transcriptional misregulation in cancer	-3.32		-6.03
Rheumatoid arthritis	-3.25		-2.36
VEGF signaling pathway	-3.20		-2.79
Pathways in cancer	-2.76		-3.87
Graft-versus-host disease	-2.54		-3.75
Neutrophil extracellular trap formation	-2.34		-5.93
Cellular senescence	-2.18		-2.49
Intestinal immune network for IgA production		-2.92	-4.23
Lipid and atherosclerosis		-2.64	-3.42
Alcoholic liver disease		-2.23	-3.45
Complement and coagulation cascades		-2.02	-3.32

Advaita iPathwayGuide, showing log(10) p-value for combined over-representation-plus-perturbation analysis of differentially expressed genes versus Vehicle group

CONCLUSIONS

- HCC tumors in a FATTY LIVERS were more resistant to drug therapy than in nonfatty livers
- The cyclophilin inhibitor, RENCOFILSTAT, combined synergistically with anti-PD1 to suppress tumor growth
- Rencofilstat altered 3-times more genes and KEGG pathways than anti-PD1 in tumors in fatty livers
- Rencofilstat + anti-PD1 IgG is a potential combination therapy for HCC in patients with fatty liver disease.