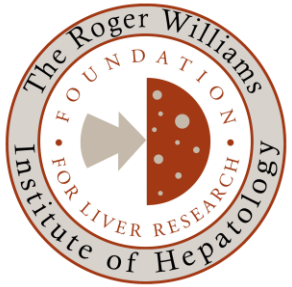
Disclosure of interest

Una Rastovic

I have nothing to declare.



2nd World Congress on Alcohol and Alcoholism

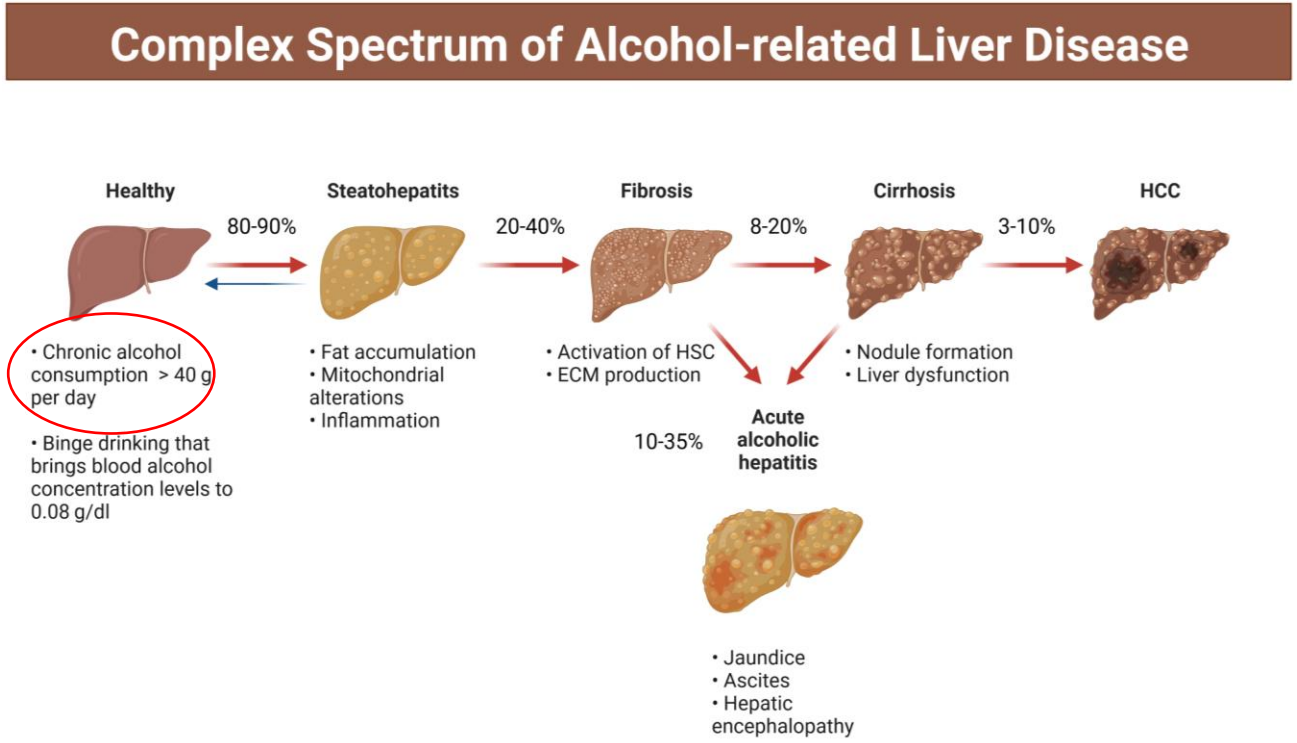
Cyclophilin inhibitor Rencofilstat as a potential therapy for Alcohol-related Liver Disease

Elena Palma, **Una Rastovic**, Sara Campinoti, Nicola Harris, Omolola Ajayi, Bruna Almeida, Tsin Shue Koay, Sandra Phillips, Karoline Lackner, Daren Ure, Melissa Preziosi, Rosa Miquel, Yoh Zen, Andreas Prachalias, Krishna Menon, Nigel Heaton, Luca Urbani, Shilpa Chokshi

Kraków, September 2022

Alcohol-related Liver Disease

- **PROBLEM**: 3 million deaths (5.3%) annually (WHO)
- **CURRENT TREATMENT**:
  - alcohol abstinence
  - interventions following a decompensating event, acute liver failure, or HCC – late-stages of ALD
- **TREATMENT OPPORTUNITY**: 30 years to intervene and prevent late-stage ALD!
- **CHALLENGE**: multiple molecular and physiological pathways disrupted

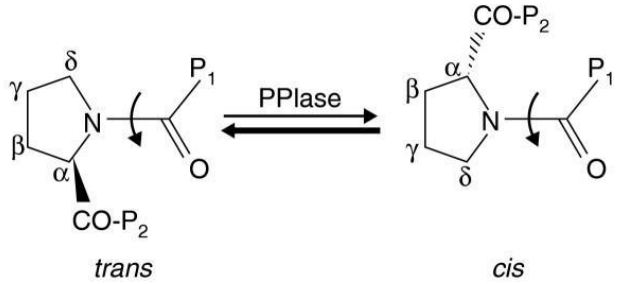


Seitz HK, Bataller R, Cortez-Pinto H, et al. 2018

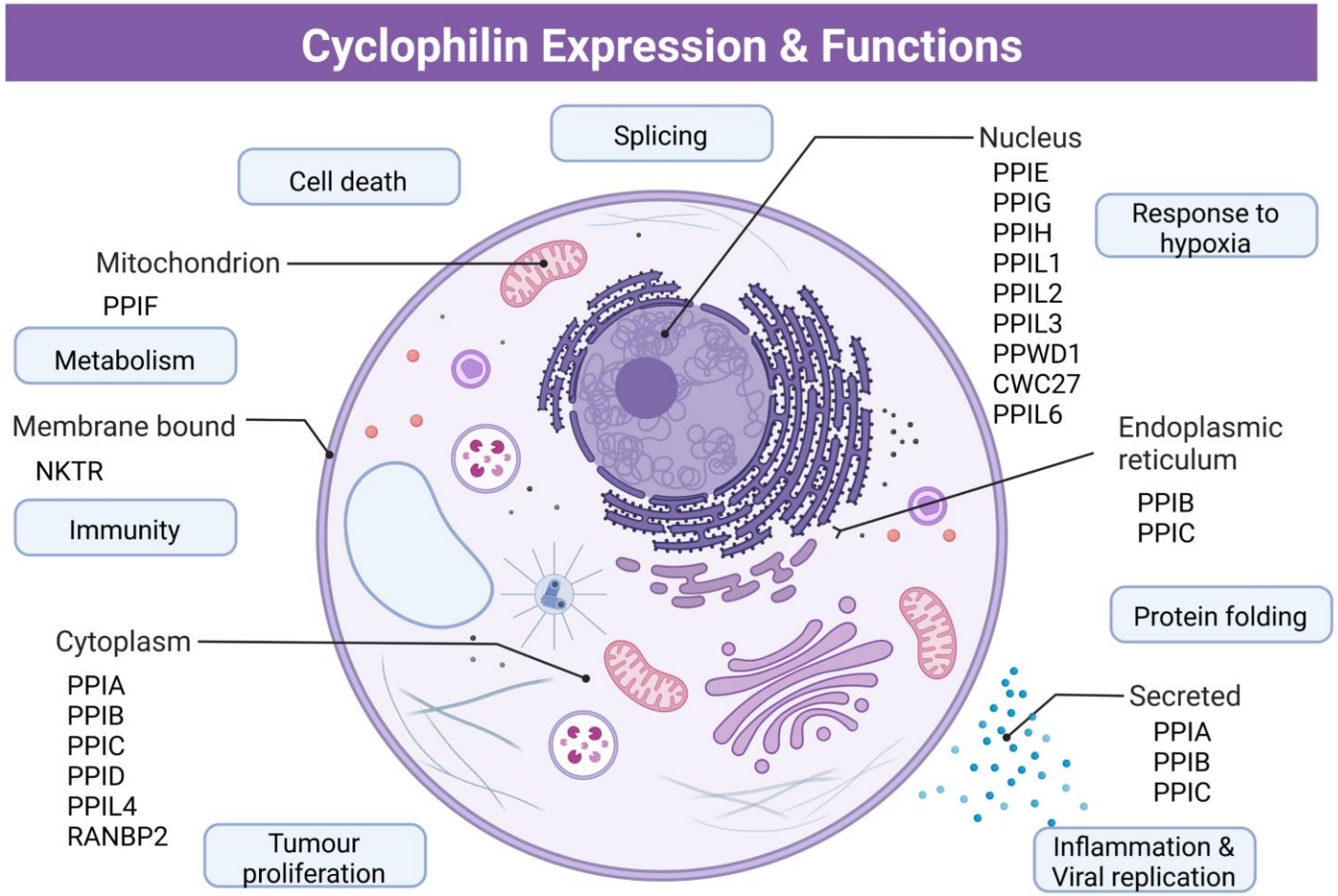
Osna NA, Donohue TM Jr, Kharbanda KK. Alcohol Res. 2017

Cyclophilins

- peptidyl-prolyl isomerase (PPIase)

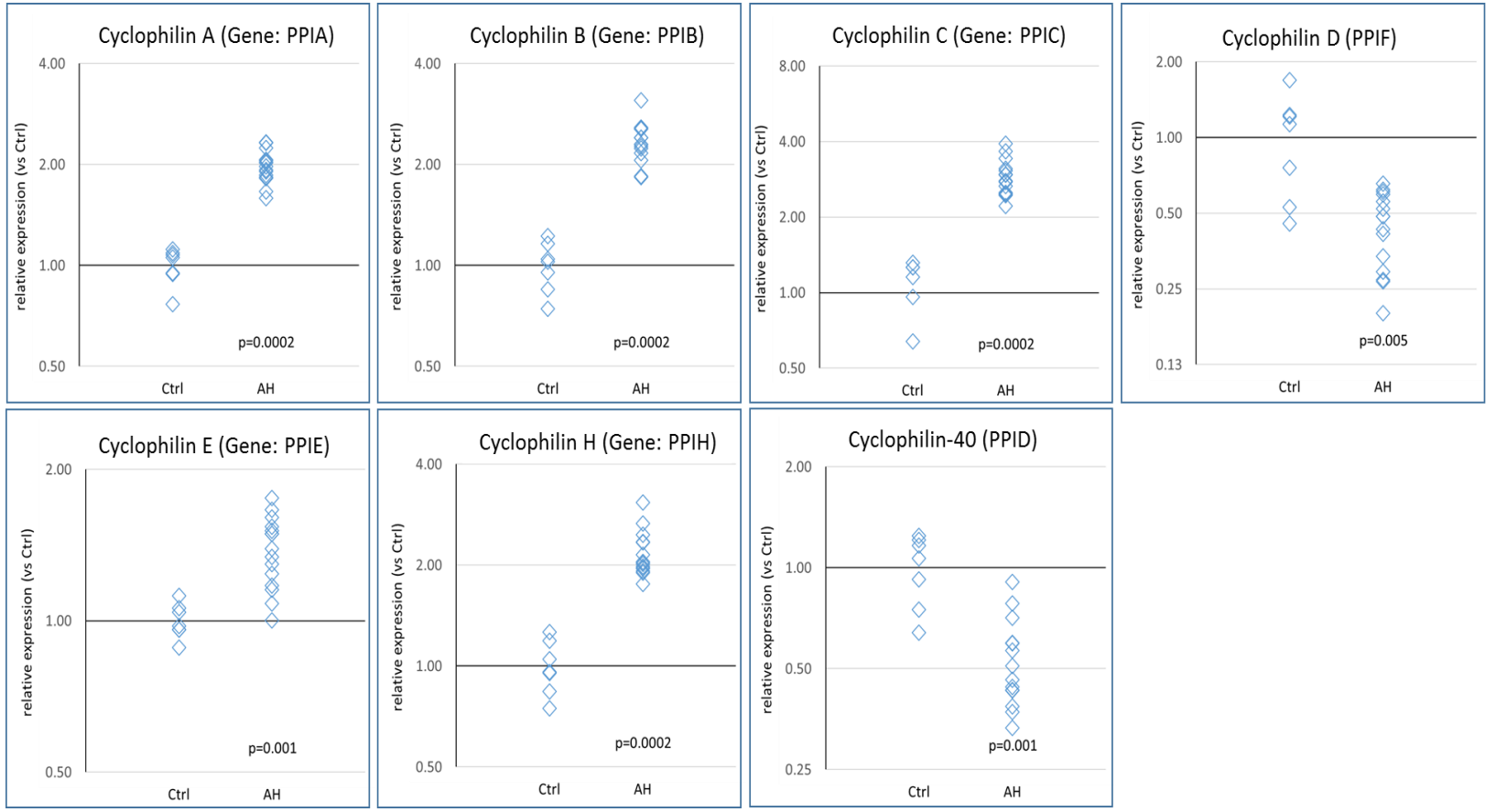


- facilitate protein folding and conformational changes -> function of the targeted proteins
- ubiquitously expressed (nucleus, ER, secreted)
- involved in different cellular processes
- elevated expression and secretion in different pathologies (*e.g.* inflammation, cancer)



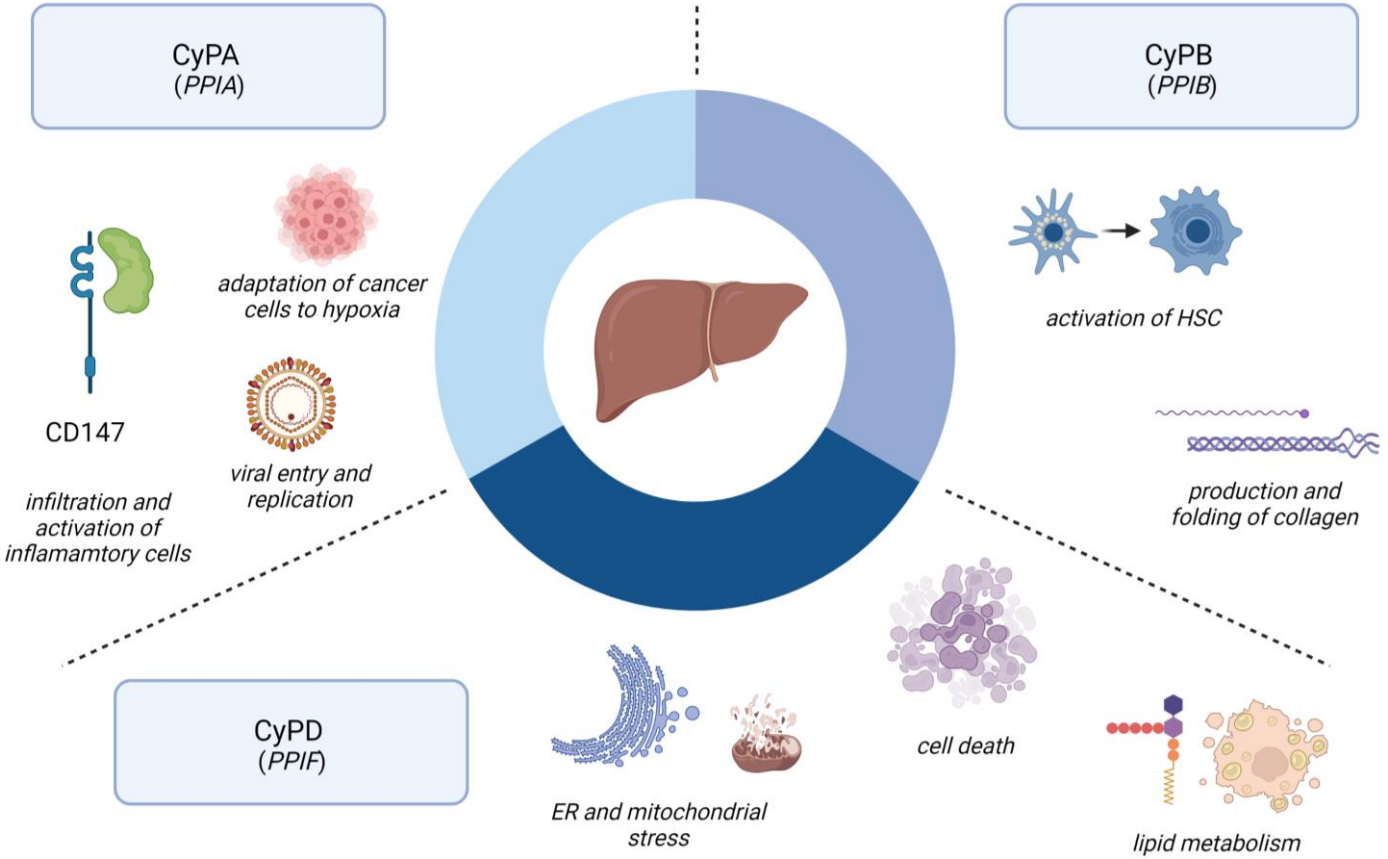
Naoumov NV. J Hepatol. 2014
Rajiv C, Davis TL. Biomolecules. 2018

Cyclophilin network is highly dysregulated in liver biopsies from patients with Alcoholic Hepatitis



DNA microarray in liver biopsies from patients with Alcoholic Hepatitis (AH, n=15) and compared to healthy controls (Ctrl, n=7)- GEO Dataset reference series GSE28619 (reanalysis of a publicly available dataset previously published by Affò S, Dominguez M, Lozano JJ, Sancho-Bru P et al. *Gut* 2013)

Role of cyclophilins in liver disease



- Effects of cyclophilin inhibition *in vitro* and in animal models
  - suppressed HBV and HCV infection and replication
  - reduced inflammation
  - suppressed collagen production and enhanced collagenase activity
  - prevented mitochondrial dysfunction and cell death
  - attenuated liver injury, and stimulated liver regeneration after massive hepatectomy

Naoumov NV. J Hepatol. 2014

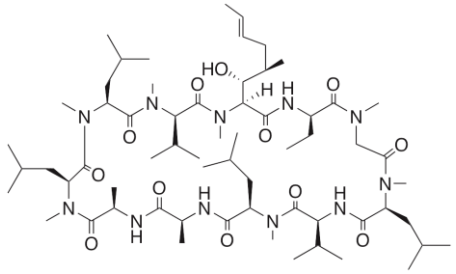
Phillips S and Chokshi S, *et al*. Gastroenterology. 2015

Bobardt M, Hansson MJ, Mayo P, *et al.* PLoS One. 2020

Ure DR, Trepanier DJ, Mayo PR, Foster RT. Expert Opin Investig Drugs. 2020.

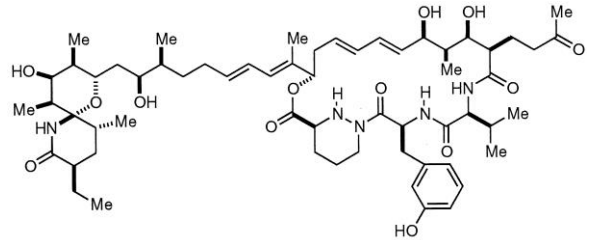
Cyclophilin inhibitors

- Cyclosporin A (CsA)



  - fungus *Tolypocladium inflatum,* 1971.
  - pancyclophilin inhibition
  - immunosupressive – therapy following transplantation
  - nonimmunosupressive derivatives: Alisporovir (DEBIO-025), NIM811, SCY-635, STG-175

- Sanglifehrins



Sanglifehrin A (SFA)

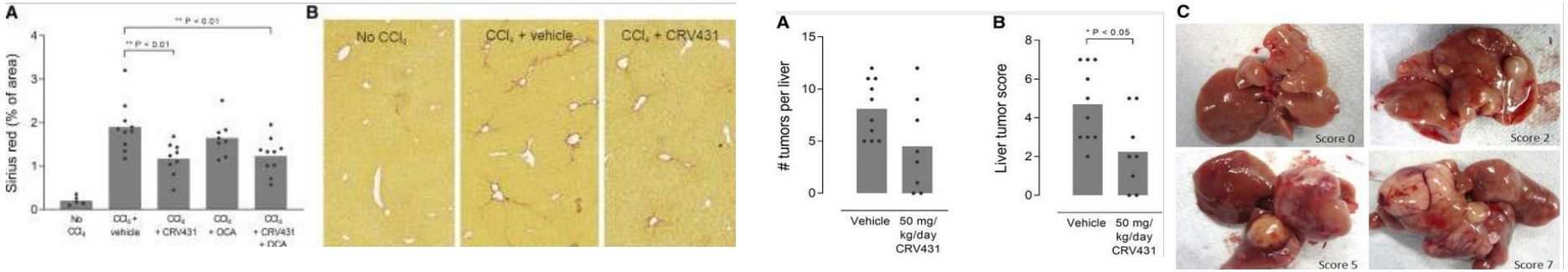
  - soil *Streptomyces*
  - pancyclophilin inhibition
  - immunosuppresive
  - nonimmunosupressive derivative: NV556

Naoumov NV. J Hepatol. 2014

Ure DR, Trepanier DJ, Mayo PR, Foster RT. Expert Opin Investig Drugs. 2020.

Rencofilstat (CRV431)

- nonimmunosupressive derivative of CsA
- potently inhibited all cyclophilin isoforms tested—A, B, D and G
- antiviral effect, decreased fibrosis and tumor development in chronic liver disease mouse models



- beneficial effect in different stages of liver disease
- Phase 2b clinical trial in NASH & Phase 2a clinical trial in HCC

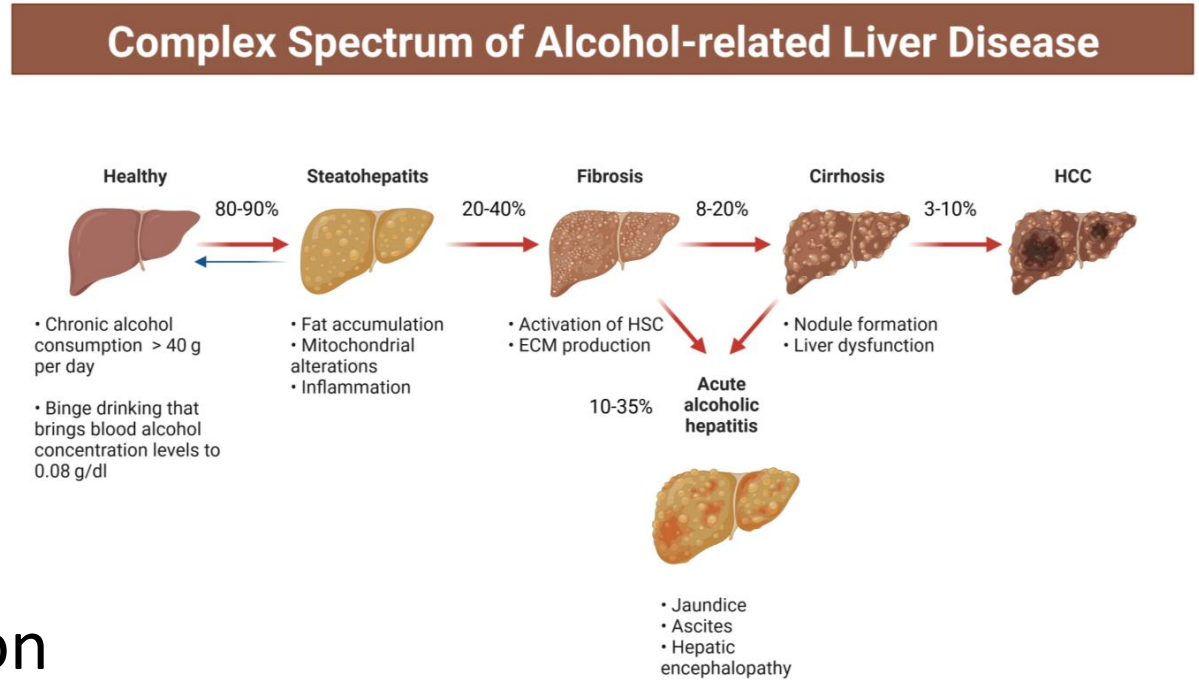
Kuo J, Bobardt M, Chatterji U, *et* al. J Pharmacol Exp Ther. 2019

Gallay P, Ure D, Bobardt M, *et.al*. PLoS One. 2019

Bobardt M, Hansson MJ, Mayo P, *et al.* PLoS One. 2020

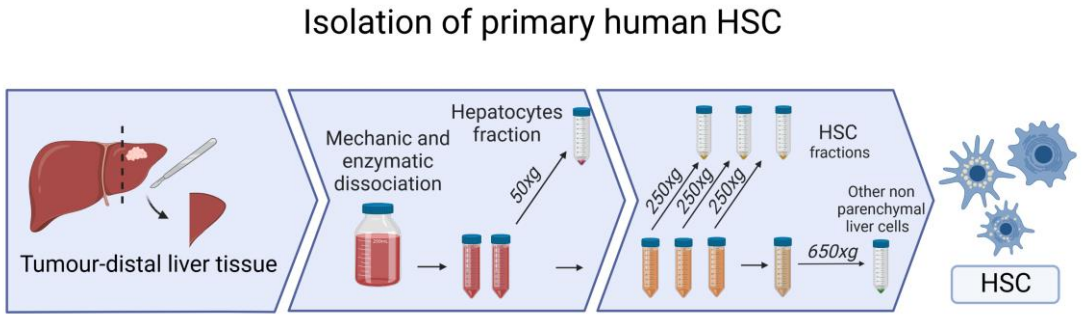
Aim

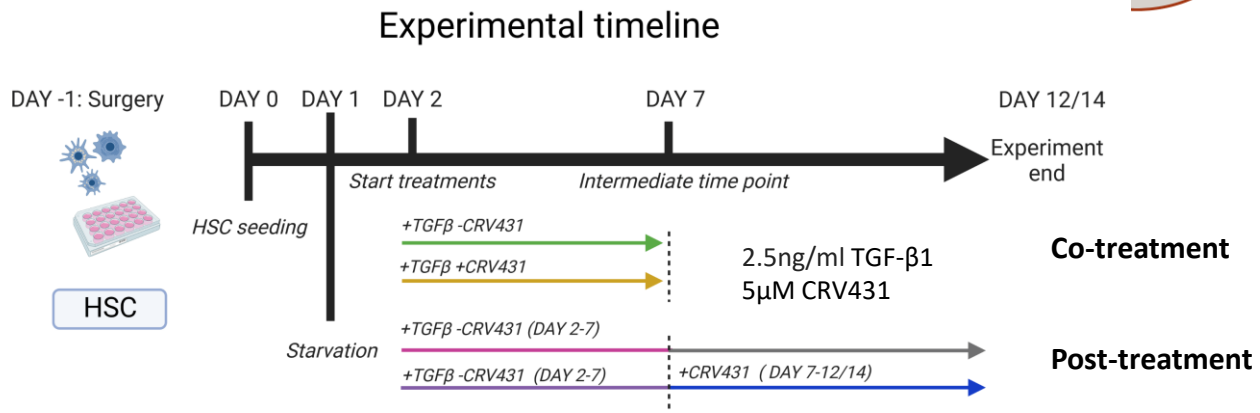
- Efficacy of CRV431 in ALD:
  - 2D model of primary human HSC
  - 3D model of human PCLS



- Fibrosis: HSC activation & ECM production
- Hepatotoxicity
- Inflammation

Methods: 2D culture of primary human HSC



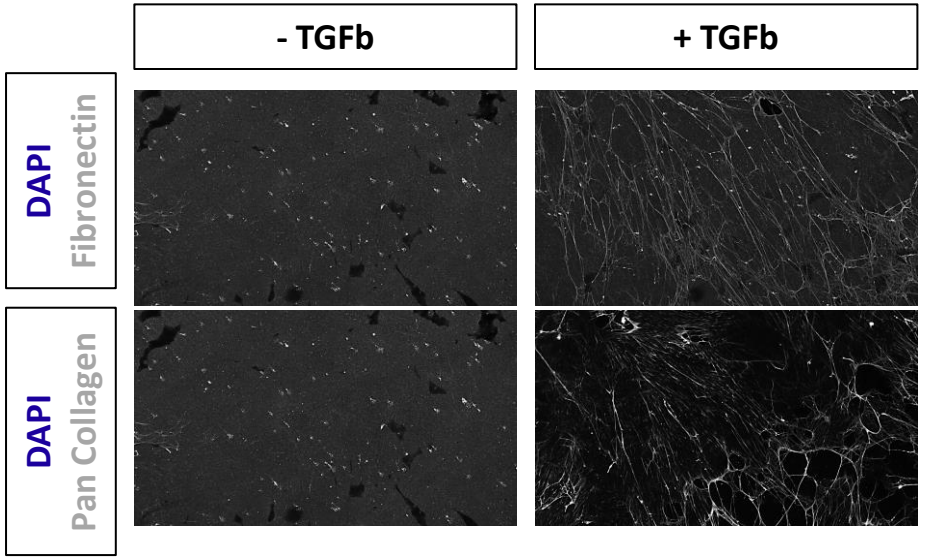


Baseline characteristics of the liver tissue donors for the production of HSC.

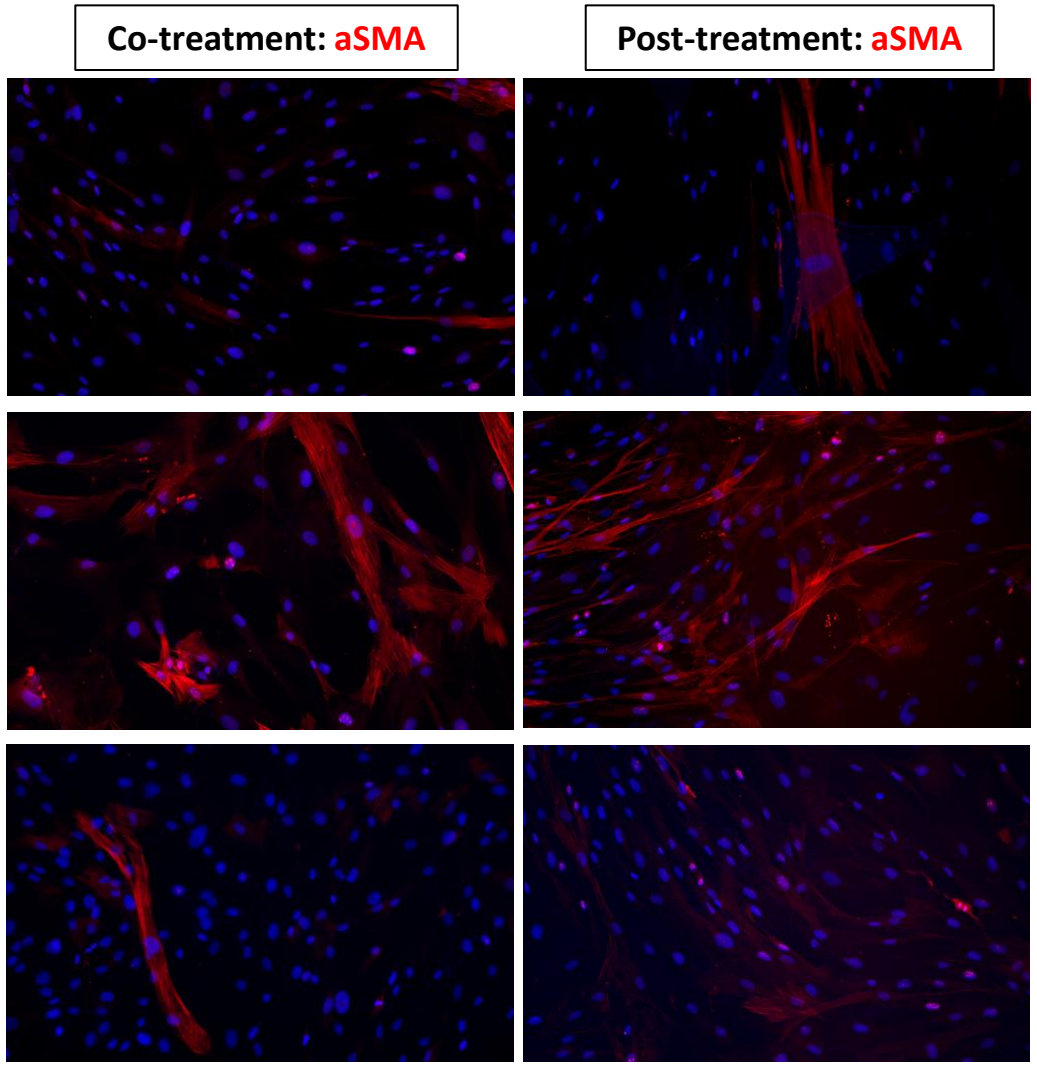
	Demographics				Background liver	Tumour		Alcohol	
SUBJECT ID	Gender	Age	Ethnicity	BMI	Fibrosis score	Aetiology	Treatment (Y/N)	current/ former	Units/ week
PCLS-152-KCH	M	40	Caucasian	29.2	F1-F2	CRLM	UA	N	UA
PCLS-156-KCH	F	69	Caucasian	17.3	F0	CRLM	Y	current	<14
PCLS-159-KCH	M	40	Asian	24.8	F1	CRLM	N	N	UA
PCLS-190-KCH	M	60	Caucasian	26.7	F0	CRLM	N	N	UA

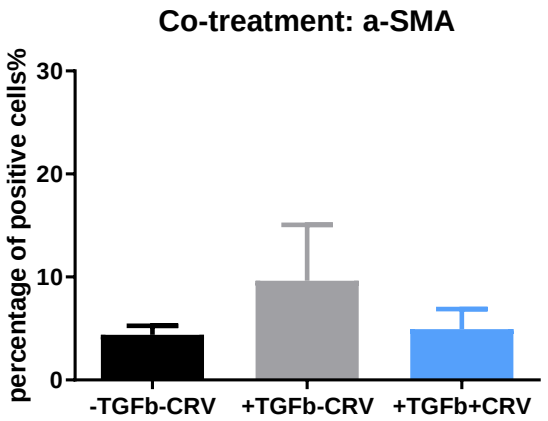
Abbreviations: BMI – body mass index, UA – unknown, CLRM – colorectal liver metastasis

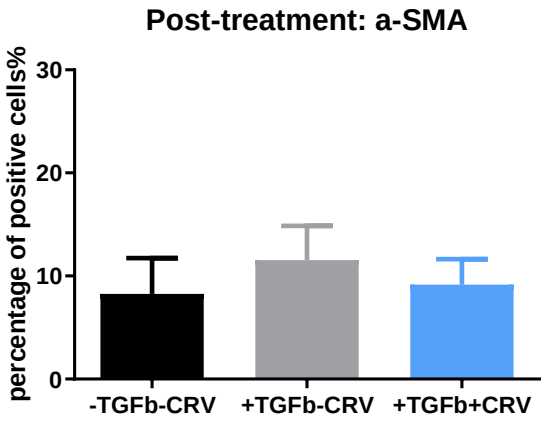
- TGF-β induces HSC to produce ECM *in vitro*

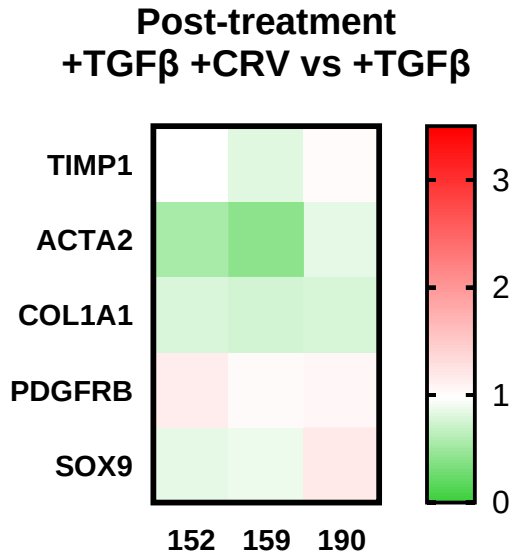


Antifibrotic in human HSC: CRV431 decreased expression of profibrogenic markers in TGFβ stimulated HSC









Heatmap showing differential gene expression of activation markers on HSC from 3 subjects treated with TGFβ alone or in combination with CRV431.

Representative images of αSMA immunostaining (in red, DAPI in blue for nuclear counter stain) on HSC159 treated with TGFβ alone or with TGFβ+CRV at time points day 7 and day 12. Quantification of αSMA positive cells (over the total of DAPI⁺ cells) in cells treated with TGFβ alone or in combination with CRV at time points day 7 and day 12. n=3 cell lines, mean ±SEM.

Thanks Dr Sara Campinoti

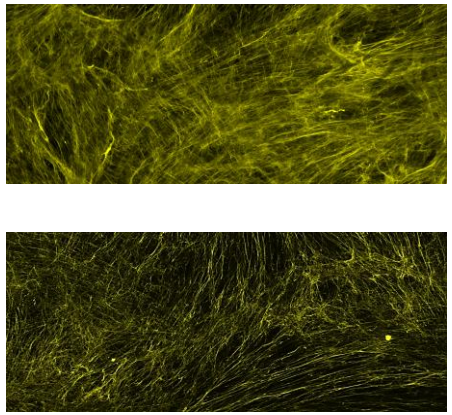
HSC

Antifibrotic in human HSC: CRV431 reduced ECM production and significantly altered the orientation of ECM fibers produced by HSC

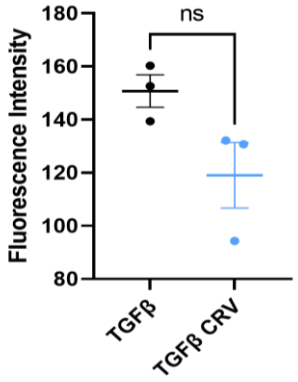
Post-treatment: Pan Collagen

+TGFβ

+TGFβ +CRV



Post-treatment:
Pan Collagen



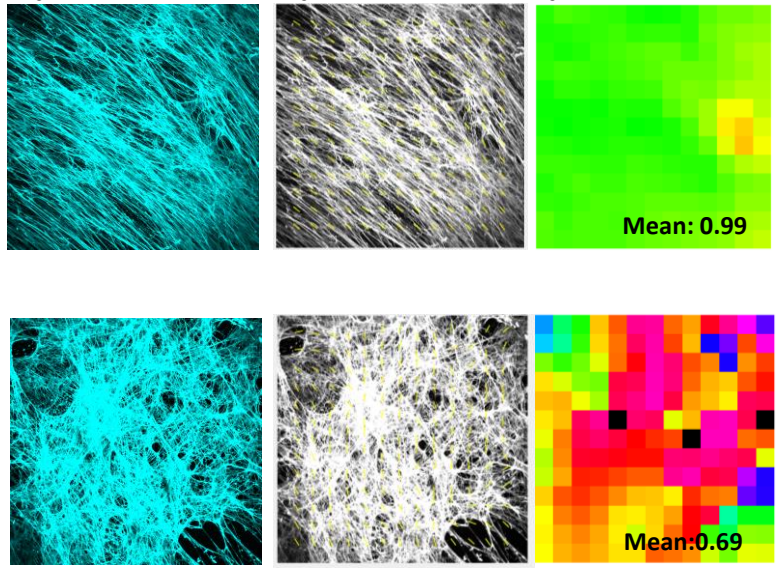
Representative IF images showing Pan-Collagen staining of ECM fibers deposited by HSC159 treated with TGFβ alone or with TGFβ+CRV. Quantification of Pan-Collagen via IF (measure of intensity of fluorescence) in HSCs treated with TGFβ or with TGFβ+CRV at the indicated timepoints. n= 3 cell lines; 6 pics/condition MEAN±SEM; statistical analysis: Wilcoxon-Mann-Whitney Test.

Co-treatment: Pan Collagen

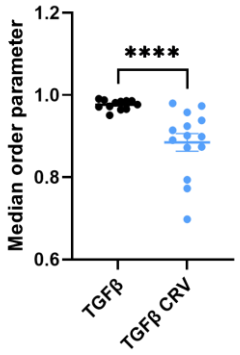
a’ a’’ a’’’

+TGFβ

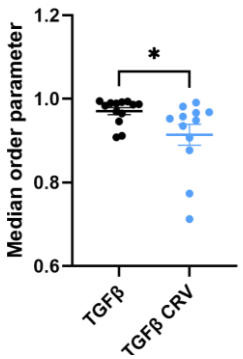
+TGFβ +CRV



Co-treatment:
Pan Collagen



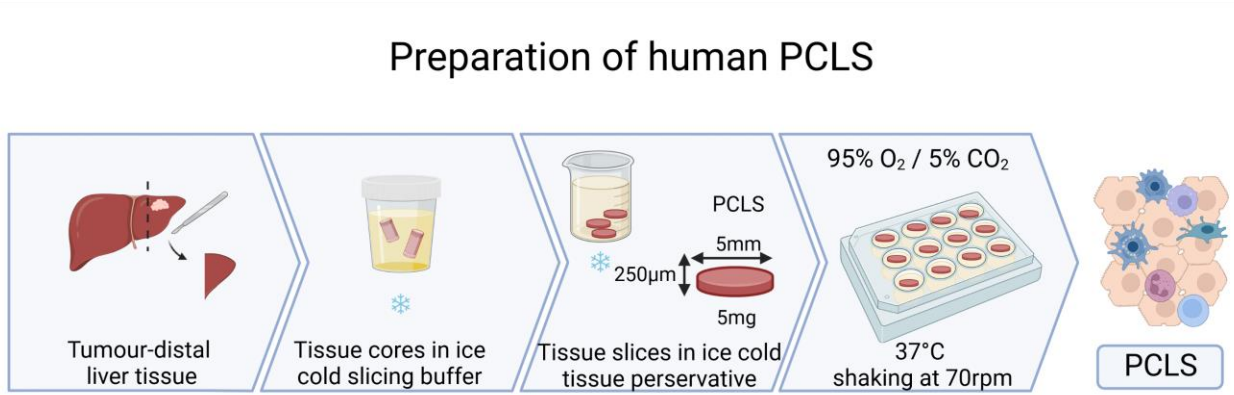
Co-treatment:
Fibronectin

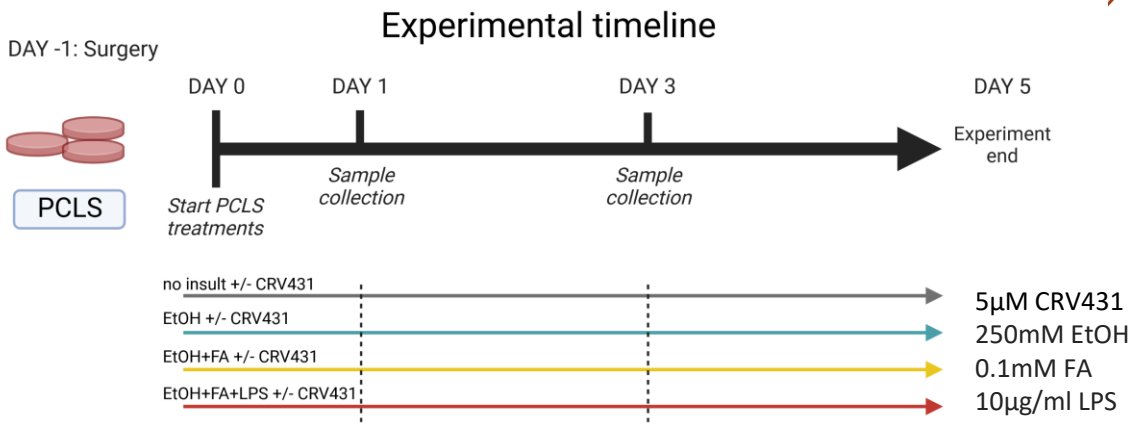


Representative images showing ECM fibers alignment study. a’, representative confocal Z-stack images showing Pan-Collagen staining of ECM fibers deposited by HSC159 treated with TGFβ alone or with TGFβ+CRV. a’’, segments (in yellow) produced by analysis software AFT – *Alignment by Fourier Transform*; each segment is oriented in the same direction as ECM fibers. a’’’, heatmap is then generated according to fibers orientations, showing diversity of neighbour fibers orientation. Mean order parameter of Pan-Collagen and Fibronectin fibres alignment in HSC159-deposited ECM after 7 days of treatment with TGFβ or TGFβ+CRV. n=13-14 pics/condition. mean±SEM; statistical analysis: Wilcoxon-Mann-Whitney Test

Thanks Dr Sara Campinoti

Methods: 3D culture of human PCLS





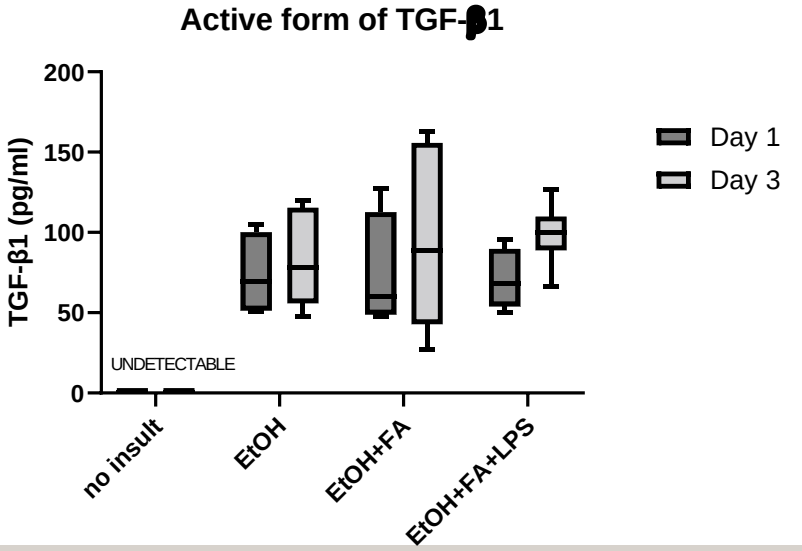
Baseline characteristics of the liver tissue donors for the production of PCLS.

	Demographics				Background liver	Tumour		Alcohol	
SUBJECT ID	Gender	Age	Ethnicity	BMI	Fibrosis score	Aetiology	Treatment (Y/N)	current/ former	Units/ week
PCLS-130-KCH	F	81	Caucasian	28.97	F1-F2	CRLM	Y	N	UA
PCLS-132-KCH	M	39	Caucasian	UA	F2-F3	CRLM	N	UA	UA
PCLS-149-KCH	F	37	Caucasian	19.36	F0	CRLM	Y	UA	UA
PCLS-152-KCH	M	40	Caucasian	29.2	F1-F2	CRLM	UA	N	UA
PCLS-156-KCH	F	69	Caucasian	17.3	F0	CRLM	Y	current	<14
PCLS-159-KCH	M	40	Asian	24.8	F1	CRLM	N	N	UA
PCLS-190-KCH	M	60	Caucasian	26.7	F0	CRLM	N	N	UA

Abbreviations: BMI – body mass index, UA – unknown, CLRM – colorectal liver metastasis

Palma E, Doornebal EJ, Chokshi S. Hepatol Int. 2019

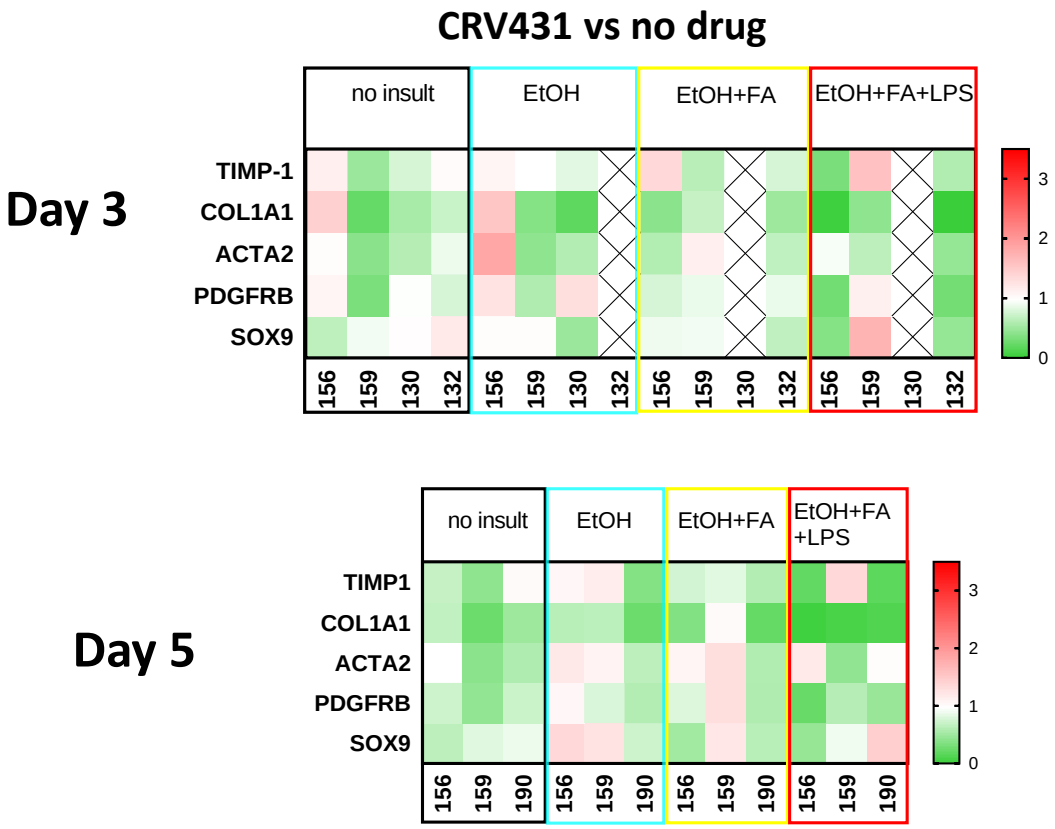
- Alcohol-induced fibrosis



PCLS

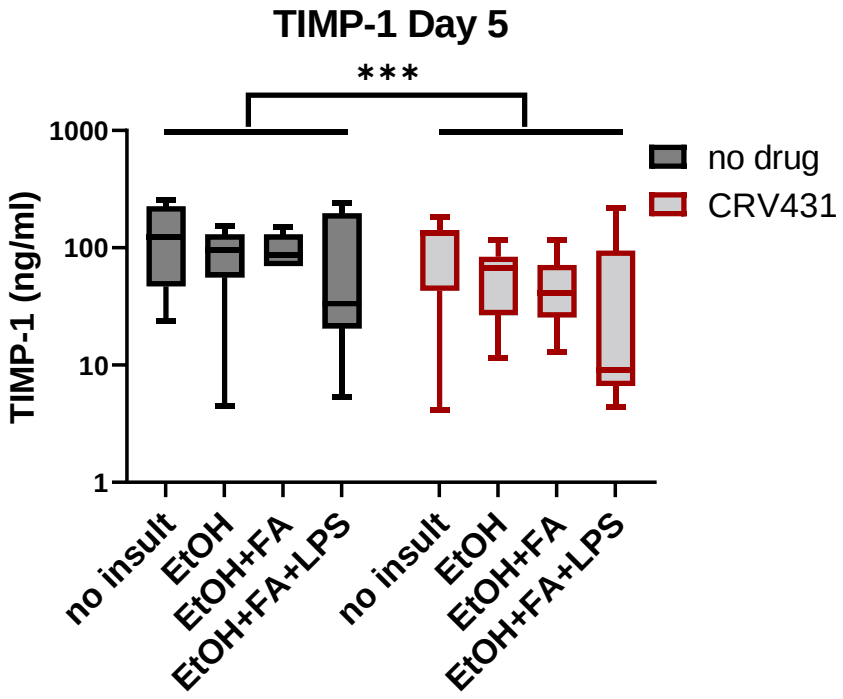
Antifibrotic in ALD: Addition of CRV431 reduced expression and secretion of profibrogenic markers in PCLS

Gene expression



Gene expression of profibrotic markers in PCLS treated with hepatotoxic insults and CRV431 measured by the Quantigene Plex Assay.

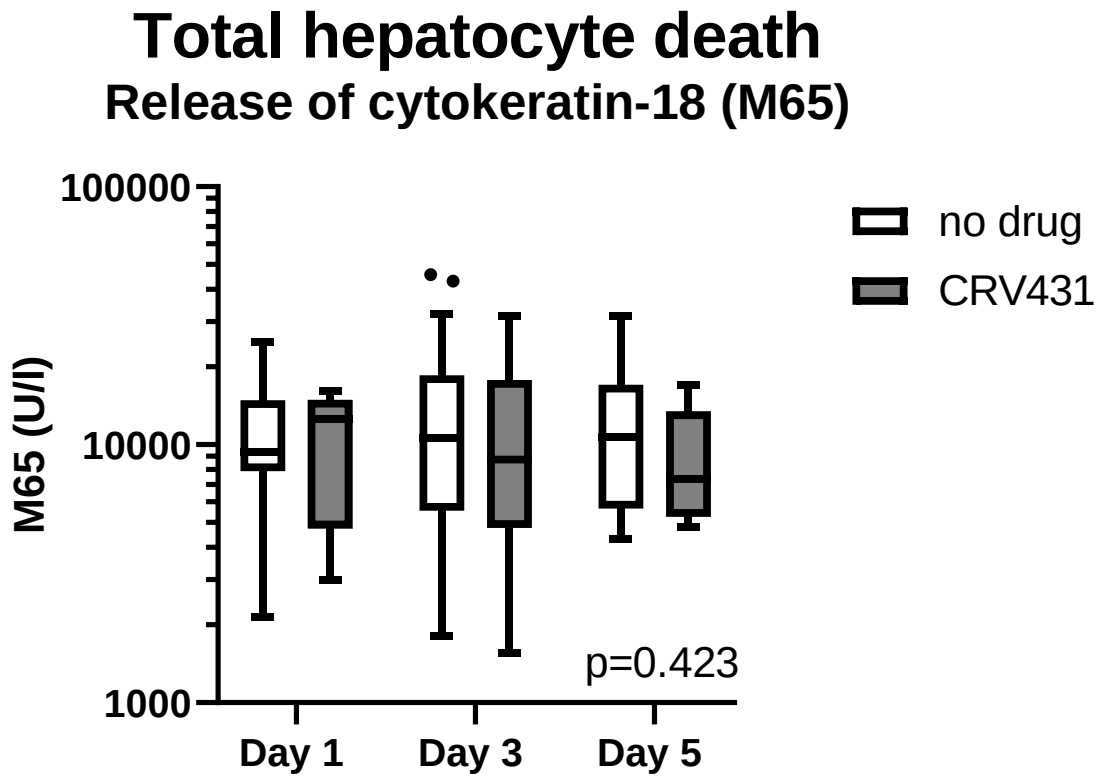
Secretion in culture supernatant



TIMP-1 release in PCLS culture supernatants. n(samples)=9, statistical analysis: 2-way ANOVA model adjusted by subject and condition, p=0.001.

PCLS

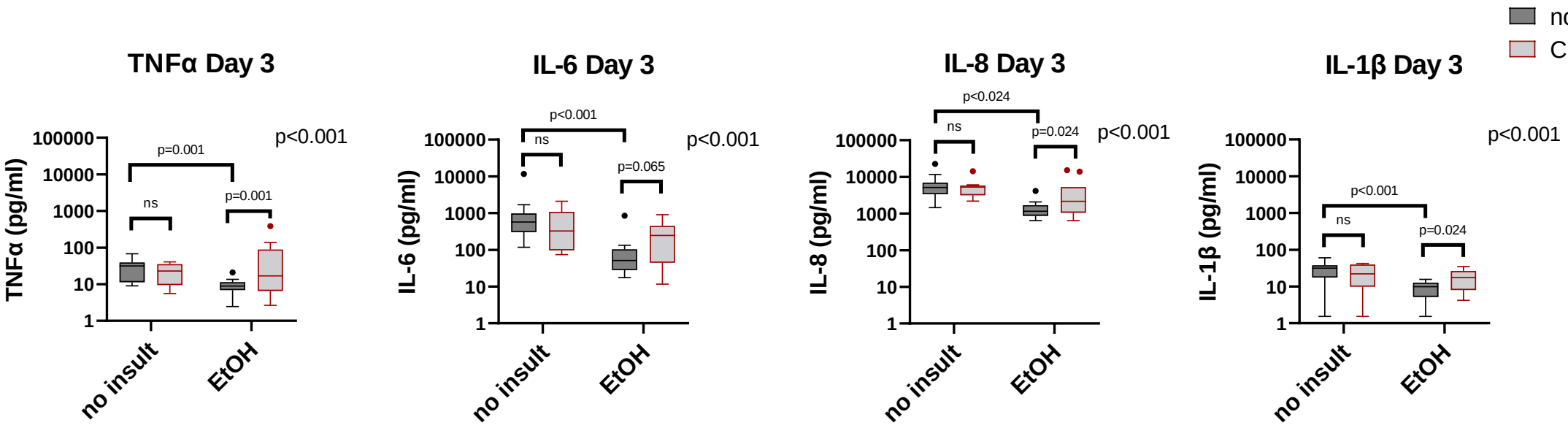
CRV431 treatment was not hepatotoxic in PCLS culture



Total hepatocyte death in PCLS measured as a release of cytokeratin-18 (M65 epitope)
n≥9, statistical analysis: 2-way ANOVA model adjusted by subject and timepoint.

PCLS

CRV431 restored a balanced cytokine profile in alcohol induced liver damage



Release of proinflammatory cytokines in PCLS culture supernatants in the presence of ethanol with and without CRV431. n(samples)=13 statistical analysis: one-way ANOVA model adjusted by subject.

LI group: Impaired immunity in ALD

Riva A,..., Chokshi S, Patel VC, Edwards LA. JHEP Rep. 2020

Riva A, Patel V, Kurioka A, Jeffery HC,...Chokshi S. Gut. 2018

Markwick LJ, Riva A,…Chokshi S. Gastroenterology. 2015

Rencofilstat (CRV431) in NASH Patients: The Phase 2a AMBITION Study

- Safe and well tolerated
- Reduction of ALT & PRO-C3: antifibrotic

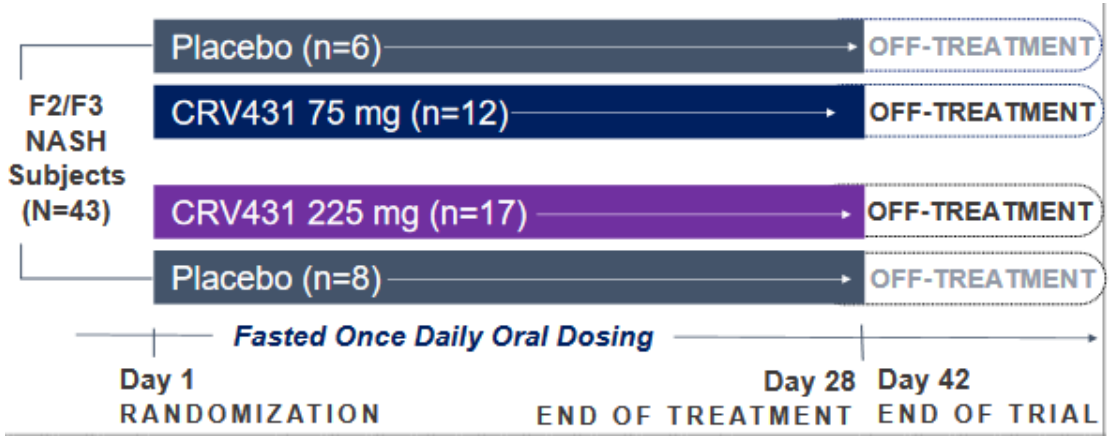


Table 2: Safety: Adverse events related to study drug

- No deaths or SAEs were reported
- Mild AEs include constipation at 75 and 225 mg
- There were 2 patients with mild diarrhea
- 225 mg: 1 report each of fatigue, lips tingling, increased weight, headache, diarrhea and 2 reports of constipation

Figure 3A: Change in ALT Baseline (ng/mL)

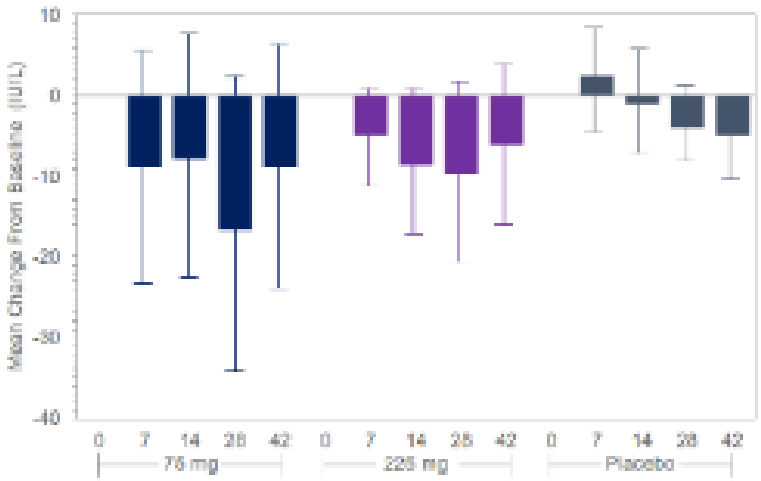
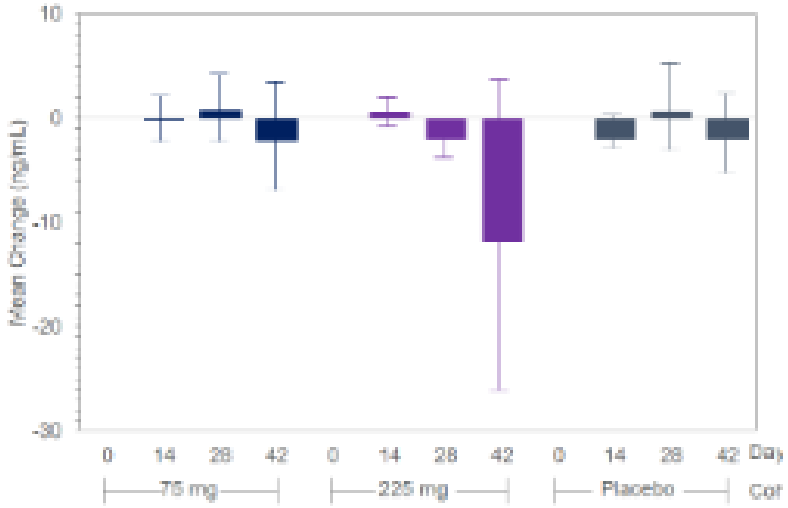


Figure 3B: Change in Pro-C3 from Baseline ≥ 17.5 ng/mL



Harrison S, Hobbs T, Mayo PR, *et al.* **NASH-TAG 2022**

Conclusions

- Cyclophilin inhibition is a promising treatment for liver diseases including ALD
- Cyclophilin inhibitor Rencofilstat (CRV431) currently in clinical development for NASH and HCC has a potential to be used as a treatment in ALD:

  - Reduced expression and secretion of profibrotic markers in primary human HSC and in alcohol-associated fibrosis in PCLS model
  - Decreased ECM production and altered the orientation of ECM fibers produced by HSC
  - Not directly hepatotoxic
  - Restored balanced cytokine profile following alcohol-induced liver damage

Acknowledgements

Liver Immunology

Dr Elena Palma

Dr Shilpa Chokshi

Nicola Harris

Ravi Jagatia

Dr Antonio Riva

Dr Sandra Phillips

Ellen Hook

Phoebe Tsou

Tsin Shue Koay

Liver Regeneration & Tissue Engineering

Dr Luca Urbani

Dr Sara Campinoti

Bruna Almedia

Lai Wei

Omlola Ajayi

Founders

King's College Hospital NHS Foundation Trust

Prof. Nigel Heaton

Prof. Yoh Zen

Dr Rosa Miquel

Mr Andreas Prachalias

Mr Krishna Menon

Ane Zamalloa

Farooq Malik

Melissa Preziosi

Marjorie Yumol

All the patients