# CRV431 Directly Targets Liver Fibrosis in Murine NASH Models and Human Liver Slices

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## BACKGROUND

CYCLOPHILINS: multi-isoform family of enzymes that control proline bond isomeric structure and in turn regulate protein conformation and function. Collagen synthesis is one function regulated by ER-resident cyclophilin B.

CRV431: a pan-cyclophilin inhibitor (nonimmunosuppressive analog of cyclosporine A) currently in a Phase 1 multiple ascending dose clinical study.

## METHODS

Cell Cultures

Procollagen and fibronectin secretion from cultured cells

Human Precision Cut Liver Slices (Fibrofind, UK)

Healthy margins of liver cancer resections. TGFβ+PDGF-BB stimulation for 3 days. Measurement of tissue RNA, secreted proteins, and Sirius Red collagen.

Animal Models

1) Carbon tetrachloride in C57BL/6 mice: 0.5 ml/kg, i.p. 2x/week, for 6 weeks

2) NASH model in C57BL/6 mice: 200 µg streptozotocin i.p. plus high fat diet (HFD)

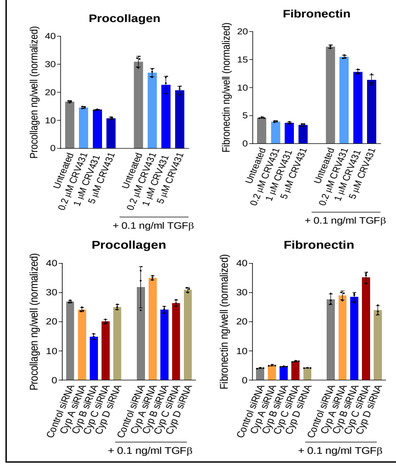
3) NASH model (Friedman) in C57BL/6 mice: Western diet + sugar-water + low-dose i.p. carbon tetrachloride ($CCl_4$)

Drug Administration in Animal Models

CRV431 (50 mg/kg/day), obeticholic acid (10 mg/kg/day), or CRV431 vehicle by oral gavage once-daily

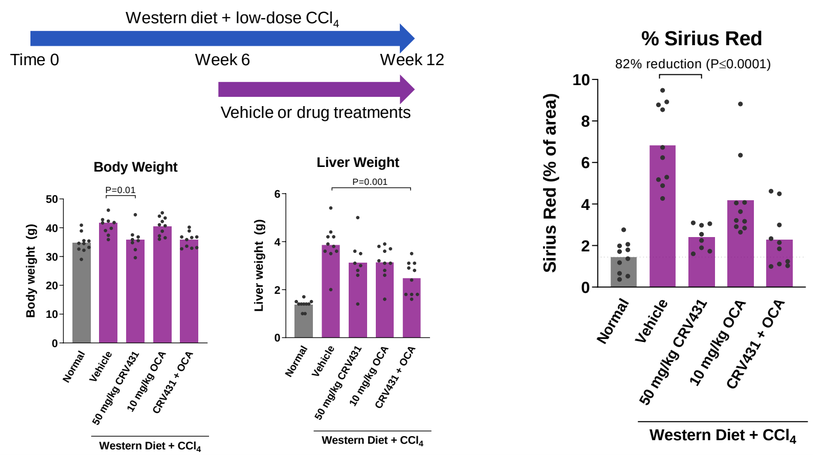
## RESULTS

### LX-2 Hepatic Stellate Cells



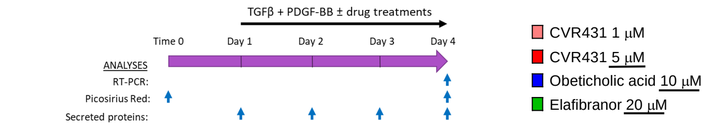

**CRV431 decreased secretion of procollagen and fibronectin in cultured LX-2 cells. A similar finding with Cyp B siRNA suggests CRV431 effect occurs through inhibition of cyclophilin B.**

### NASH Mouse Model (Friedman model; Western diet + low-dose $CCl_4$)

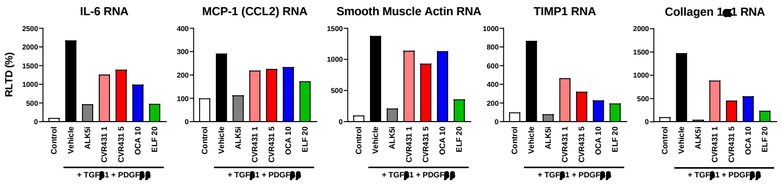



**CRV431 alone or in combination with obeticholic acid for 6 weeks significantly decreased liver fibrosis (Sirius Red staining).**

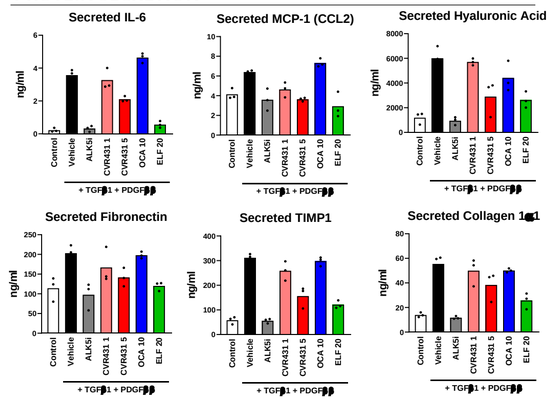
### Human Precision Cut Liver Slices (Fibrofind, Newcastle, UK)

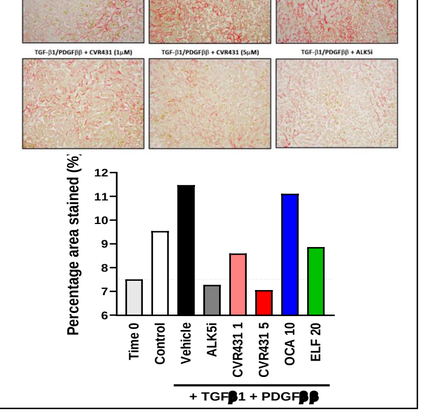



**GENE EXPRESSION**



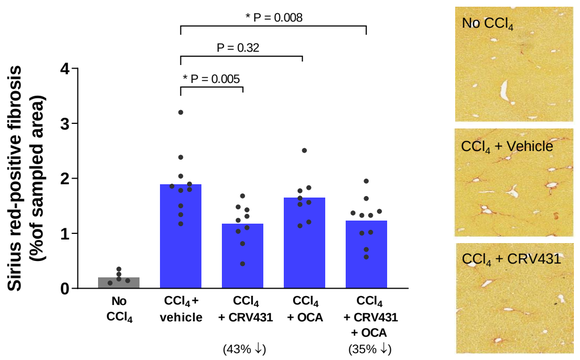

**PROTEIN SECRETION**



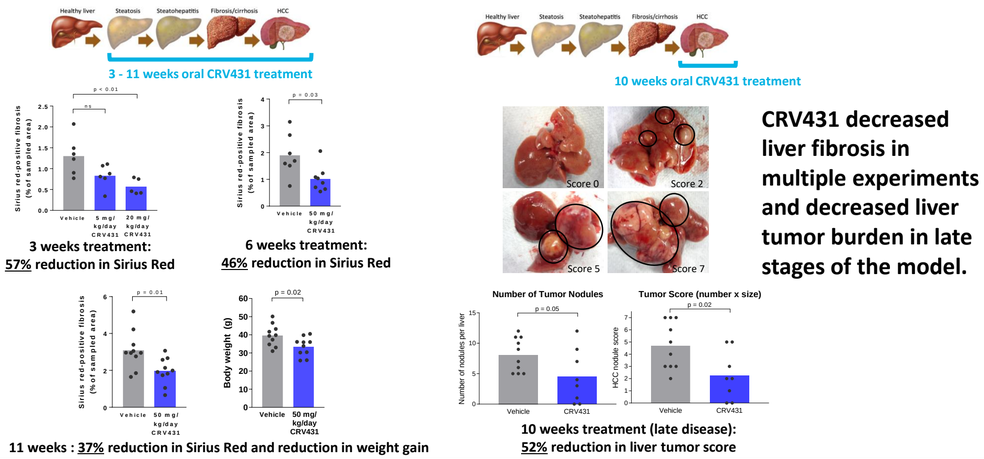

**CRV431 decreased markers of inflammation and fibrosis (RNA and protein) and completely blocked fibrosis induced by TGFβ+PDGF-BB stimulation**

### Carbon Tetrachloride Mouse Model




**CRV431 for 6 weeks, alone or with obeticholic acid (OCA), decreased $CCl_4$-induced liver fibrosis**

### NASH Mouse Model (streptozotocin + high fat diet)




**CRV431 decreased liver fibrosis in multiple experiments and decreased liver tumor burden in late stages of the model.**

## CONCLUSIONS

- CRV431 directly reduced collagen production in cultured cells and reduced liver fibrosis in multiple models, which likely occurred in part through inhibition of ER-resident cyclophilin B.
- CRV431 also demonstrated anti-inflammatory and anti-tumor effects which may occur through other cyclophilin-dependent mechanisms.