# CRV431: An Optimized Cyclophilin Inhibitor with Multiple Anti-HBV Activities, High Selectivity Index, and Synergy with CMX157

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## INTRODUCTION

**Novel drug combinations targeting multiple HBV activities are needed to eliminate HBV.**

### CRV431

- $CRV_{431}$, a novel cyclophilin inhibitor ("CPI"), designed to be layered on top of HBV therapeutic drugs including NUC backbone

### CMX157

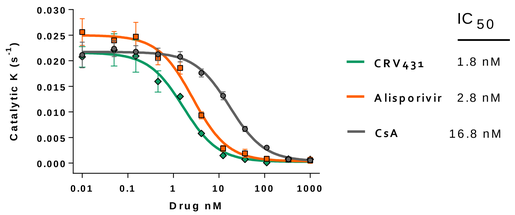
- $CMX_{157}$, a nucleotide ("NUC") analogue of tenofovir, designed to reduce viral load and serve as backbone HBV therapy

### IDEAL THERAPEUTIC DRUG COMBINATION

- Additive to synergistic, with wide *in vitro* Selective Index ("SI") to optimally position combination for wide Therapeutic Index ("TI") in clinic, while reducing exposures compared with monotherapy
- Targets multiple stages of the HBV life cycle

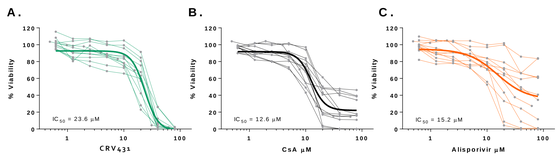
## CRV431 NON-VIRAL EFFECTS

### CRV431 is a highly potent cyclophilin inhibitor




Cyclophilin A inhibition was assessed with the chymotrypsin-coupled isomerase assay using 10 nM recombinant cyclophilin A and succinyl-AAPF-pNA peptide substrate.

### CRV431 has less cytotoxicity than other cyclophilin inhibitors




| Primary Human Cells | Human Cell Lines |
|---|---|
| Renal epithelial | HepaRG hepatocyte |
| Bronchial smooth muscle | Jurkat lymphocyte |
| Dermal fibroblast | |
| Umbilical vein endothelial | |

Each curve = one experiment in one cell type

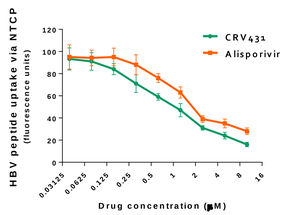
Cells plated in triplicate at moderate density and treated for 3 days with compounds or DMSO vehicle. Viability assessed with resazurin-based (metabolic) assay and normalized to DMSO.

**COMPARISONS**

- In vitro anti-HBV $IC_{50} \approx$ 0.03 - 0.7 µM
- Anticipated plasma $C_{max}$ and $C_{trough} \approx$ 2 µM and 0.7 µM (based on alisporivir clinical efficacy)

## ANTI-HBV ACTIVITIES

### CRV431 inhibits NTCP-mediated HBV entry

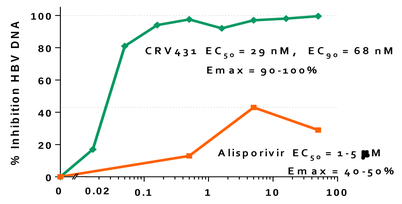



Other studies show that NTCP inhibition by cyclosporin analogs occurs through a cyclophilin-independent mechanism.

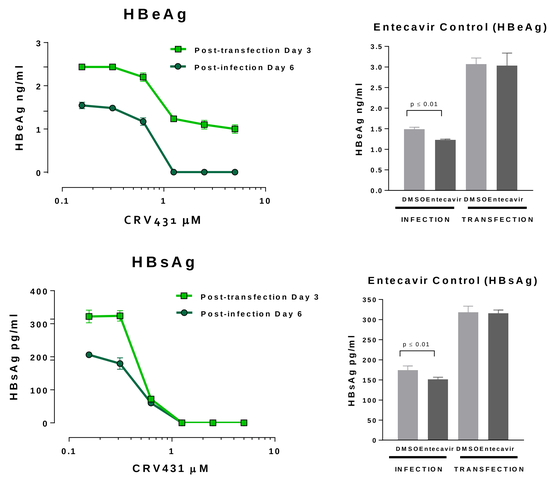
$CRV_{431}$ $IC_{50} \approx 1$ µM

Uptake of HBVpreS peptide-FITC by NTCP-Huh7 cells (generous gift from Dr. Urban).

### CRV431 blocks HBV DNA replication in AD38 cells



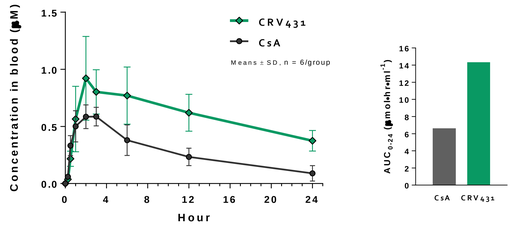

### CRV431 blocks HBeAg and HBsAg production and/or secretion in infected and transfected cells, unlike entecavir




NTCP-Huh7 (infection assays) and Huh7 (transfection assays). CRV431 treatment begun prior to infection and transfection. Measure extracellular HBsAg by ELISA on Day 6 post-infection or Day 3 post-transfection.

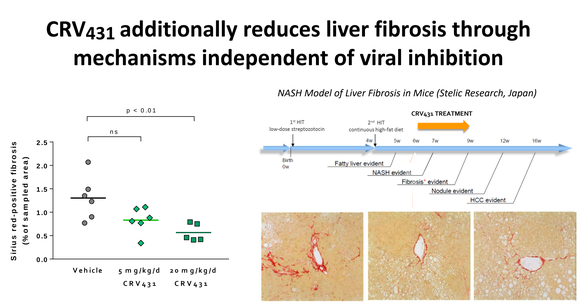
## CRV431 *IN VIVO*

### CRV431 is suitable for oral dosing




Single oral dose of $CRV_{431}$ at 10 mg/kg in 6 male and 6 female rats. Male and female rats showed similar responses.

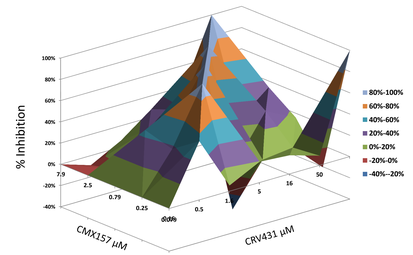
### CRV431 additionally reduces liver fibrosis through mechanisms independent of viral inhibition




Cyclophilins are implicated in fibrotic mechanisms, such as collagen maturation, degradation, and hepatic stellate cell activation.

## CRV431 + CMX157 COMBINATION

### CRV431 inhibits HBV synergystically with CMX157




AD38 cells treated with multiple combinations of CRV431 and CMX157 for 5 days. Measurement of intracellular HBV DNA.

## SELECTIVE INDEX MODEL

**$CRV_{431}$ Selective Index in AD38 cells (HBV DNA)**

**= $CC_{50}/IC_{50}$**

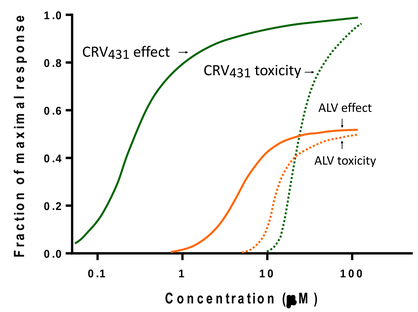
**= 23,600 nM / 29 nM (AD38)**

**= 814**

**Selective index depends on cell type and marker (e.g. HBV DNA, HBeAg, HBsAg)**

**Optimized selective index of $CRV_{431}$ may provide for enhanced clinical utility.**

**Possible utility may be further enhanced by combination therapy with $CMX_{157}$.**




## CONCLUSIONS

- $CRV_{431}$ has a wide SI, as defined by the ratio of $CC_{50}$ to $IC_{50}$ *in vitro*
- The SI of $CRV_{431}$ is the widest of any known CPI, potentially offering a wide TI in patients
- Thus far, $CRV_{431}$ addresses many of the identified endpoints relevant to HBV drug therapy including:
  - Reduction of HBV DNA
  - Suppression of HBeAg and HBsAg
  - Inhibition of viral entry *via* NTCP
- $CRV_{431}$, in combination with CMX157, is synergistic (reduction of HBV DNA)
- $CRV_{431}$ has potential beneficial effects on progression of liver fibrosis