CRV431: Multiple Therapeutic Actions *In Vitro* and *In Vivo*

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**INTRODUCTION**

CRV431 is a novel non-immunosuppressive analog of cyclosporine A (CsA) targeting cyclophilins. Cyclophilins are cellular host proteins that participate in the HBV life cycle, and their inhibition has been proposed as a treatment for chronic HBV. The molecular mechanism(s) of action of cyclophilin inhibitors in HBV infection are not well defined. Herein, we describe actions of CRV431 both in *vitro* and in *vivo* aimed at elucidating possible mechanisms of action.

**CRV431 Reduces HBV DNA, HBSAg, and HBeAg in Cellular Models**

HBV-integrated cell lines: Reduction in HBV DNA

**MODEL:** AD38 and DE519 cell lines were induced (tetracycline removed) and treated with CRV431 or the tenofovir pro-drug, TXL, for 6 days.

**HBV infection and transfection: Reductions in HBSAg + HBeAg**

**MODEL:** CRV431 or entecavir was applied for up to 6 days to NTCP-Huh7 cells infected with HBV or Huh7 cells transfected with HBV plasmid.

**CRV431 Blocks HBSAg-Cyclophilin Binding**

**Pull-Down Assays:** Tagged wild-type or isomerase-dead (H126Q) cyclophilin A (CypA) was used to capture purified His-HBSAg or native HBSAg in lysates from HBV plasmid-transfected cells, followed by Western blot detection.

**CRV431 Blocks HBx-Cyclophilin Binding**

**Pull-Down Assays:** GST-CypA was used to capture purified His-HBx or HA-HBx in lysates from HA-HBx plasmid-transfected cells, followed by Western blot detection.

**CRV431 Has a High Selective Index**

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**CRV431 Inhibits NTCP in HBV + HDV Co-infection**

CRV431 inhibited HBV and HDV coinfection *in vitro*, consistent with blocking NTCP-mediated virus uptake.

**MODEL:** HBV and HDV co-infection of stably transfected NTCP- HepG2 cells. CRV431 applied during 4-hr virus inoculation and for 6 days post-inoculation.

**CRV431 Reduces cccDNA and pgRNA in Primary Human Hepatocytes**

**MODEL:** PHH treated with CRV431 starting 1 day after the start of HBV infection. Assay HBV DNA, cccDNA, and pgRNA at Days 4 and 7.

**CRV431 Reduces HBV DNA and HBSAg in HBV Transgenic Mice**

**MODEL:** CRV431 and/or the tenofovir pro-drug, TXL, were administered orally to HBV transgenic mice for 16 days, followed by measurement of HBV in livers.

**CRV431 Reduces Liver Fibrosis in a NASH Model**

**MODEL:** CRV431 was administered orally to “NASH” mice for 21 days (Weeks 6-9), followed by histological quantitation of fibrosis.

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**CONCLUSIONS**

- CRV431 reduces HBV DNA, cccDNA, pgRNA, HBSAg, and HBeAg in a variety of cellular models.
- CRV431 blocks cyclophilin A binding to HBSAg and HBx, which may be part of the mechanism(s) of action.
- CRV431 has anti-HBV activity and anti-fibrotic activity in mouse models.
- CRV431 has the potential for multiple, therapeutic effects in hepatitis B patients with a favorable toxicity profile.