CRV431 and CMX157 (TXL; tenofovir exalidex): Anti-HBV combination effects *in vitro* between a cyclophilin inhibitor and a nucleotide prodrug

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INTRODUCTION

It is expected that a cure for HBV will require drug combinations that interact at more than one stage of viral replication and propagation. Our lead drug, tenofovir exalidex (TXL; formerly CMX157), a tenofovir (TFV) prodrug, is a novel lipid acyclic nucleoside (NUC) phosphonate designed to deliver high intrahepatic concentrations of TFV, while minimizing off-target effects caused by high levels of circulating TFV. CRV431, our earlier-stage molecule, is a host targeting antiviral that inhibits cyclophilins, namely cyclophilin A (cypA), a peptidyl prolyl isomerase. As a cypA inhibitor, CRV431 reduces HBV DNA, suppresses HBsAg, inhibits viral uptake via NTCP and, more recently, has been shown to impede HBx-cypA binding.

AIM

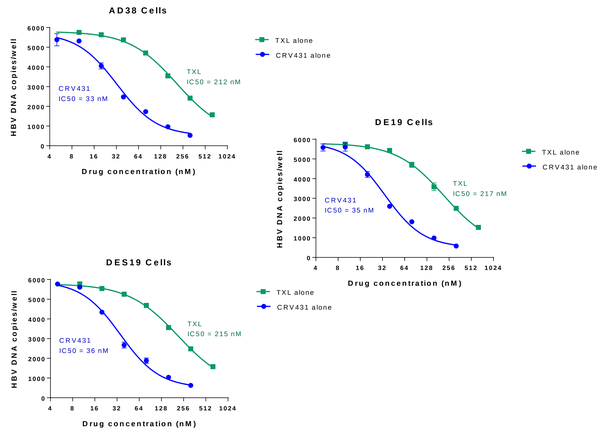
The aim of the current study was to investigate the combination anti-HBV effects of CMX157 and CRV431 by measuring HBV DNA levels.

METHOD

The current study measured inhibition of intracellular HBV DNA at concentrations of CRV431 ranging from 0-320 nM alone, and in combination with CMX157 ranging from 0-640 nM. Both drugs were tested *in vitro* in AD38, DE19, and DES19 cells. Studies were each conducted twice, in triplicate wells, using DMSO as control. Drug concentration versus effect was evaluated using Prichard-Shipman MacSynergy. Additionally, CRV431 cytotoxicity was examined in a number of primary human cells and cell lines, utilizing DMSO and 0.5% saponin as negative and positive controls, respectively, comparing cyclosporine, alisporivir, and sanglifehrin A, to confirm cell viability in the assays.

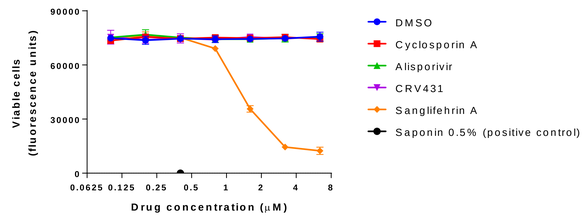
RESULTS

CRV431 and TXL independently inhibit HBV replication in HepAD38, DE19, and DES19 cells



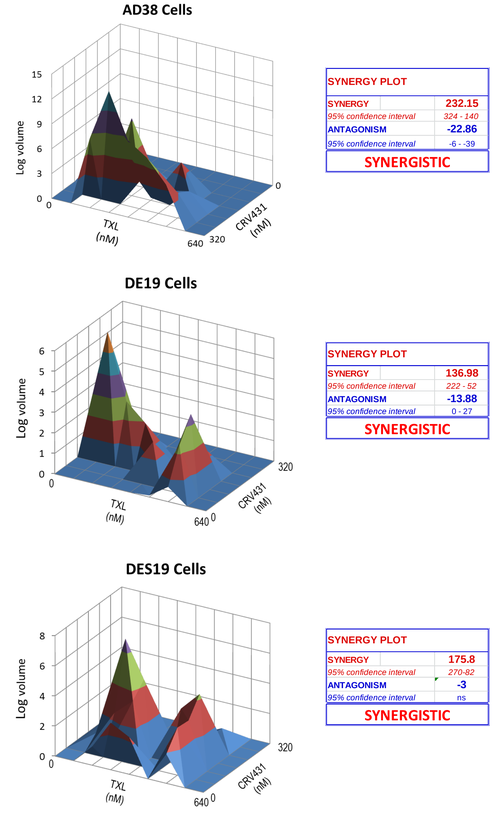
HepAD38, HepDE19 (HBeAg-deficient) and HepDES19 (HBeAg- and HBsAg-deficient) cells were incubated for two weeks with tetracycline to suppress HBV replication. Tetracycline was then removed and cells treated with increasing concentrations of the indicated drugs. After 3 days, medium was removed and replaced with fresh medium and drugs. After 3 days (6 days of drug exposure), intracellular HBV DNA was purified and quantified by qPCR. HBV DNA data are expressed as number of intracellular HBV DNA copies per well in triplicate.

CRV431 and other cyclosporins are much less cytotoxic than sanglifehrin A (alternate class of cyclophilin inhibitor)



HepG2 cells in 96-well plates were treated with the indicated compounds for 3 days. Cell viability was then assessed with the Live/Dead Assay involving flow cytometric measurement of calcein-AM and the DNA-binding ethidium homodimer.

Combination treatment with CRV431 and TXL inhibits HBV synergistically (Prichard-Shipman MacSynergyII)



SYNERGISM/ANTAGONISM

0-25 = additivity	25-50 = mild synergism	50-100 = moderate synergism	>100 = strong synergy
(log volume < 2)	*(log volume = 2-5)*	*(log volume = 5-9)*	*(log volume > 9)*

HepAD38, HepDE19 and HepDES19 cells (triplicate) were treated with increasing concentrations of the two indicated drugs. Antagonistic, additive and synergistic effects were analyzed by quantification of intracellular HBV DNA by qPCR and analysis by the MacSynergyII program. This program is based on the Bliss independence model that is defined by the following equation: Exy = Ex + Ey − (Ex × Ey), where Exy is the additive effect of drugs x and y as predicted by their individual effects, Ex and Ey.

CONCLUSIONS

CRV431 and CMX157, tested in combination, represents a viable therapeutic drug strategy towards the cure of HBV. This strategy exploits the complementary modes of action of the two drugs, which allows for suppression of HBV DNA, HBsAg, HBeAg, inhibition of viral entry, and blocking of cypclophilin A binding to HBx. The complementary actions of CRV431 with CMX157 may reasonably extend to drugs with other modes of activity including, for example, core inhibitors.

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REFERENCES

Gallay PA et al. The novel cyclophilin inhibitor CPI-431-32 concurrently blocks HCV and HIV-1 infections via a similar mechanism of action. *PLOS One 2015; 10(8):e0134707*

Phillips S et al. Alisporivir inhibition of hepatocyte cyclophilins reduces HBV replication and hepatitis B surface antigen production. *Gastroenterology 2015; 148:403-414.*

Shimura S et al. Cyclosporin derivatives inhibit hepatitis B virus entry without interfering with NTCP transporter activity. *J Hepatol 2017; 66:685-692*

Prichard-Shipman MacSynergyII – manual and spreadsheet
https://www.uab.edu/medicine/peds/macsynergy

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