Assessing the in vitro anti-HBV activity of combinations including CRV431, TXL and prototype core protein assembly modulators

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INTRODUCTION

A functional cure for chronic HBV infection will likely require drug combinations targeting the viral life cycle at multiple, complementary stages. CRV431, a cyclophilin inhibitor, blocks HBV interactions with host cyclophilins (cyp) essential for viral replication and chronicity. TXL, a novel tenofovir (TFV) prodrug in clinical development, inhibits HBV polymerase and is designed to deliver high intra-hepatic tenofovir (TFV) concentrations. BAY41-4109 (heteroaryldihydropyrimidine, HAP) and DVR-56 (sulfamoylbenzamide) are both prototype core protein assembly modulators (CpAMs) which block several HBV replication steps, including pre-genomic RNA packaging, misdirection of core protein assembly and cccDNA formation. While HAPs, such as Bay 41-4109, misdirect capsid assembly to form non-capsid polymers of core proteins,[2] all other reported chemotypes of CpAMs, including DVR-56, induce the formation of variable sizes of capsids devoid of viral pgRNA and DNA polymerase. [1, 2, 3] *In vitro* studies will help identify potential future therapeutic combinations to progress into clinical development.

AIM

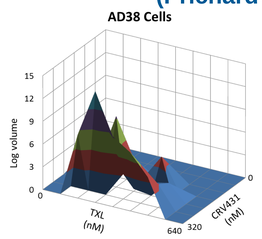
Core protein assembly modulators (CpAMs) block several HBV replication steps. A direct acting antiviral, TXL and host targeting cyclophilin inhibitor, CRV431, along with CpAMs could prove to be a therapeutic option in the future. We selected two CpAMs , each with a different mechanism of action, and reflecting two different biological outcomes. The aim of our current study was to investigate the antiviral activity combinations of TXL, CRV431, and prototype CpAMs (BAY41-4109, DVR-56) by measuring HBV DNA levels *in vitro*.

METHOD

HepAD38cells (triplicate) were treated with increasing concentrations of compounds., tested two at a time. Antagonistic, additive and synergistic effects were analyzed by quantification of intracellular/extracellular HBV DNA by qPCR and analysis by the MacSynergyII program. The compound test concentrations are as follows: CRV431 0-1000 nM, TXL 0-25 nM, DVR-56 0-250 nM and BAY41-4109 0-250 nM.

RESULTS

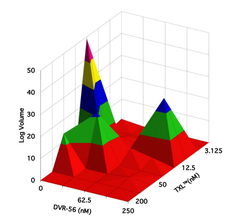
Previously Reported Combination Treatment with CRV431 and TXL Inhibits HBV Synergistically[4] (Prichard-Shipman MacSynergyII)[5]



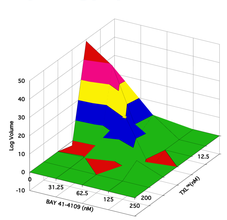
TXL/CRV431 Synergy Plot	
Synergy	232.15
95% confidence interval	324 - 140
Antagonism	-22.86
95% confidence interval	-6 - -39
STRONG SYNERGY	

SYNERGY/ANTAGONISM			
0-25 = Additivity	25-50 = Mild synergy	50-100 = Moderate synergy	>100 = Strong Synergy
(log volume < 2)	(log volume = 2-5)	(log volume = 5-9)	(log volume > 9)

Combination Treatment with TXL and DVR-56 or BAY 41-4109 Inhibits HBV Synergistically (Prichard-Shipman MacSynergyII)

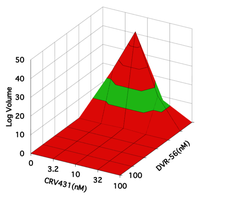


TXL/DVR-56 Synergy Plot	
Synergy	224.78
95% confidence interval	310 - 139
Antagonism	-4.62
95% confidence interval	-2 - -8
STRONG SYNERGY	

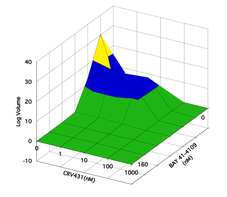


TXL/BAY41-4109 Synergy Plot	
Synergy	217.76
95% confidence interval	251 - 184
Antagonism	-42.13
95% confidence interval	-29 - -55
STRONG SYNERGY	

Combination Treatment with CRV431 and DVR-56 or BAY 41-4109 Inhibits HBV Synergistically (Prichard-Shipman MacSynergyII)



CRV431/DVR-56 Synergy Plot	
Synergy	198.54
95% confidence interval	270 - 127
Antagonism	-260.68
95% confidence interval	*not significant*
STRONG SYNERGY	



CRV431/BAY41-4109 Synergy Plot	
Synergy	96.36
95% confidence interval	98-95
Antagonism	-14.53
95% confidence interval	-7 - -22
MODERATE SYNERGY	

RESULTS AND CONCLUSIONS

- HBV cure will require an armamentarium that not only targets multiple steps of the viral life cycle but must be complementary in mechanisms of action.
- To date, nucleos(t)ide therapy has been extensively clinically proven to reduce high patient viremia and will continue to be the backbone of future HBV therapy.
- CRV431 has multiple modes of action that reduce or interact with HBV proteins (HBsAg, HBeAg, HBx, HBV DNA, pgRNA).
- Combination scores using TXL or CRV431 with a prototype CpAM rated their collective anti-HBV activity from moderate synergy to strong synergy.
  - TXL/DVR-56 = 224.78
  - TXL/BAY41-4109 = 217.76
  - CVR431/DVR-56 = 198.54
  - CVR431/BAY41-4109 = 96.36
- All scores calculated by MacSynergy II revealed no antagonism in the combinations towards lowering *in vitro* HBV DNA levels.
- *In vitro* synergy experiments may prove to be a useful tool in guiding combination therapies to cure chronic Hepatitis B.
- Studying complementary modes of action moves us closer in our efforts to find a cure for HBV.

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[4]**R. Foster et al.** CRV431 and TXL(tenofovir exalidex): Anti-HBV combination effects in vitro between a cyclophilin inhibitor and a nucleoside prodrug, ILC 2017 abstract #180.

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

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