REP 2139 targets the Hepatitis Delta Virus (HDV) ribonucleoprotein (RNP) and exerts a direct antiviral effect on HDV replication

Simone FONTE [1,2], Marie Laure PLISSONNIER [1,2], Maud MICHELET [1,2], Matthieu BLANCHET [3,4], Patrick LABONTE' [4], Andrew VAILLANT [3], Massimo LEVRERO [1,2,5]

[1] Hepatology Institute, Lyon, France; [2] Cancer Research Center of Lyon (CRCL), INSERM U1052, CNRS UMR5286, Lyon, France; [3] Replicor Inc., Montreal, Canada; [4] INRS – Institut Armand Frappier, Institut National de la Recherche Scientifique, Laval, Canada; [5] Department of Hepatology, Hôpital Croix-Rousse, Hospices Civils de Lyon, Lyon, France; [6] University of Lyon Claude Bernard 1 (UCLB1), Lyon, France

Introduction and objectives

The nucleic acid polymer (NAP) REP 2139 has multiple molecular mechanisms: 1) targeting the HSP40 chaperone DNAJB12 to block HBV subviral particles assembly and secretion (Boulon et al, Hepatology (S1) 2020; Angelo et al, HBV Meeting 2023), an effect which also blocks HDV ribonuclear protein (RNP) envelopment and HDV secretion from infected cell; 2) direct binding to S-HDAg and L-HDAg (Shamur et al, Hepatology (S1) 2017). A phase II study (Bazinet et al, Lancet GastroHep 2017) and current compassionate use of REP 2139 in HBV/ HDV infection have shown a more robust and early response toward HDV RNA as compared to HBsAg, suggesting a second direct acting antiviral mechanism. Here we have investigated the direct REP 2139 antiviral activity in relevant HDV cell infection models in vitro.

Methods

REP 2139 endosomal release in vitro was restored by UNC 7938 (Blanchet, Antiviral Res 2019). Clinical supply of REP 2139-Mg (lot FAB-22-0001) was used for dosing in HDV infected (10 ge/cell) HepG2-NTCP cells and primary human hepatocytes (PHH) and in Huh7-END cells stably replicating HDV (Ni Y, SciRep 2019). Intracellular HDV viral genome levels were assessed by qRT-PCR. HDV RNA and Hepatitis Delta Antigen (HDAg) association to form the HDV ribonucleoprotein (HDV RNP) was monitored by anti-HDAg RNA immunoprecipitation (RIP) followed by HDV qRT-PCR (Abeywickrama-Samarakoon N, Nat Comms 2020).

Results

1. A single dose of REP 2139-Mg reduced intracellular HDV viral genome levels by ~1 log10 in HepG2-NTCP, PHH cells and Huh7-END at 400nM, 600 nM and 600nM respectively.

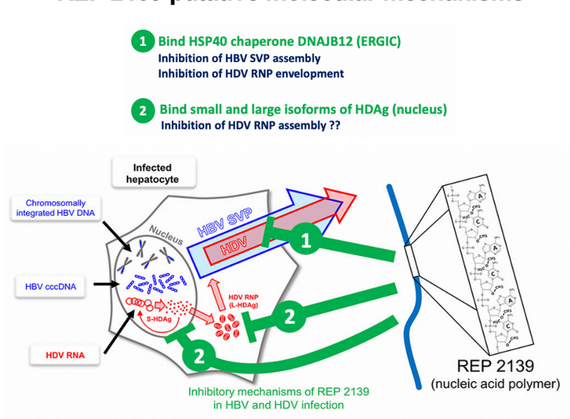
2. Loss of antiviral activity at higher doses could be recovered by increasing UNC 7938 concentration, indicating that REP 2139 endosomal release is influenced by both REP 2139 endosomal concentration and UNC 7938 dosing.

3. REP 2139-Mg reduces both genomic (≈1Log) and antigenomic (≈1.5Log) HDV RNA.

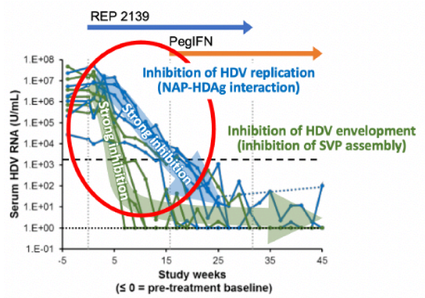
4. A single REP 2139-Mg dose also reduced the intranuclear association of HDV RNA with HDAg by ~60% (@ 600nM) in HepG2-NTCP and by 65% (@ 400nM), without changing the HDAg protein levels.

5. REP 2139-Mg inhibits HDV replication in HBV-HDV coinfected PHHs. We confirm in HBV-HDV coinfected PHHs that REP 2139 inhibits HBsAg secretion without affecting intracellular pgRNA levels or HBeAg secretion.

REP 2139 putative molecular mechanisms



REP2139 in HDV patients

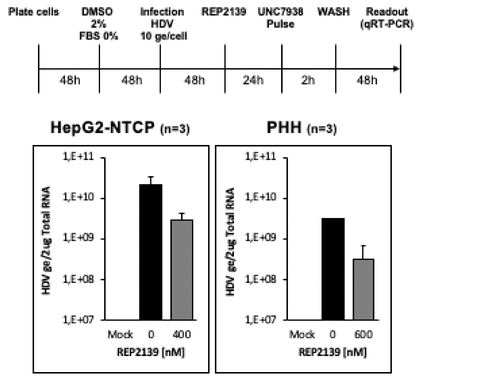


The effect on HDV RNA before PEG-IFN is started

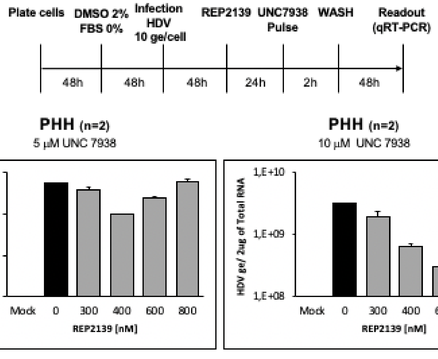
Conclusions

REP 2139 has a direct acting antiviral effect against HDV RNA replication which may involve blocking HDV RNA interaction with HDAg during HDV RNP morphogenesis. These antiviral effects bear further investigation and may explain the more rapid decline of HDV RNA versus HBsAg in human studies.

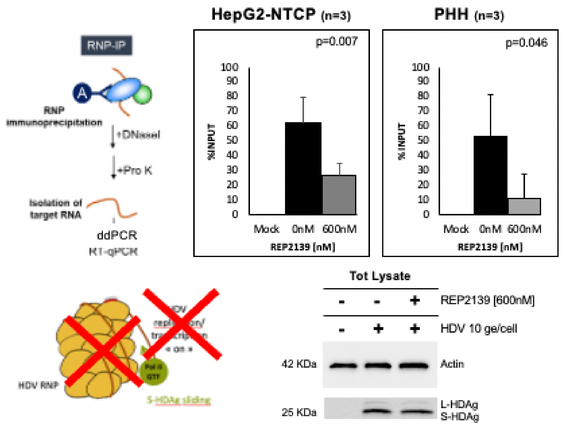
Results 1.1 A single dose of REP 2139-Mg reduces intracellular HDV viral genome levels by ~1 log_{10} in HepG2-NTCP and PHH cells



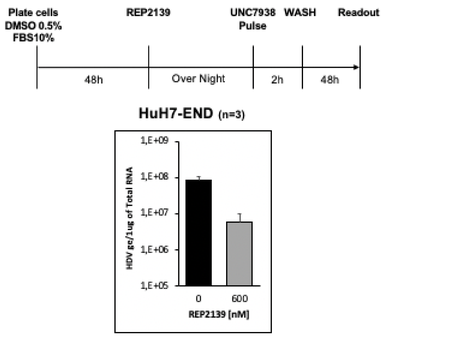
Results 2. REP2139-Mg inhibits the HDV replication in a dose-dependent manner. At higher dosage it accumulates in the endosomes and requires higher concentration of UN7938 to be released



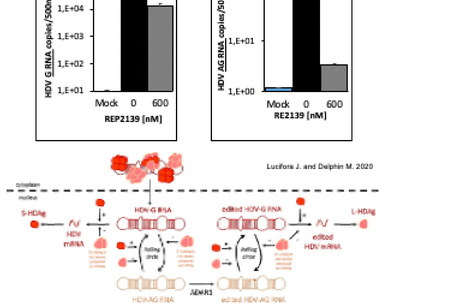
Results 4. A single REP 2139-Mg dose also reduced the intranuclear association of HDV RNA with HDAg by ~60% in HepG2-NTCP and by 80% in PHH, **without changing the HDAg protein levels**.



Results 1.2 A single dose of REP 2139-Mg reduces intracellular HDV viral genome levels by ~1 log_{10} also in the stable HBD replicating cells Huh7-END cell line that supports a complete HDV viral cycle and secretes HDV infectious virions



Results 3. REP 2139-Mg reduces both genomic (≈1Log) and antigenomic (≈1.5Log) HDV RNA



Results 5. REP 2139-Mg inhibits HDV replication in HBV-HDV coinfected PHHs. We confirm in HBV-HDV coinfected PHHs that REP 2139 inhibits HBsAg secretion without affecting intracellular pgRNA levels or HBeAg secretion