Massimo LEVRERO 3.07.2024

# Mode of action of Nucleic Acid Polymers (NAPs) in HDV infection

*Lyon Hepatology Institute, Lyon, France*
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*University of Lyon Claude Bernard 1 (UCLB1), Lyon, France*

Disclosures : Gilead, AbbVie, MSD, Inventiva, Roche, Ipsen, CLS-Behring

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RB-RNA

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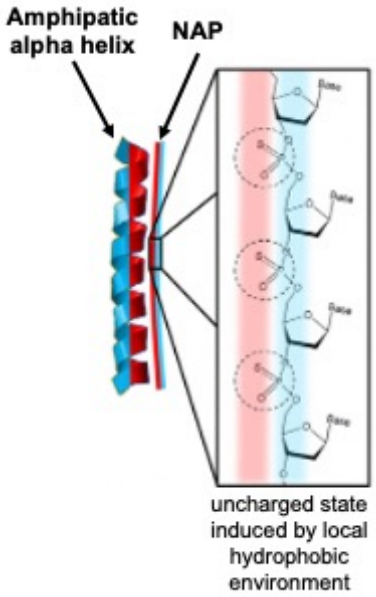
# Nucleic Acid Polymers (NAPs) and REP 2139

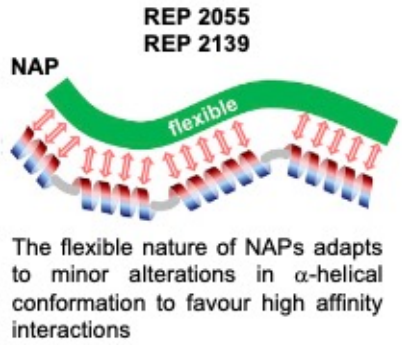
***Nucleic acid polymers*** (***NAPs***) act as broad-spectrum antiviral compounds against diverse enveloped viruses and other infectious agents.

The activity of all NAPs is **sequence independent** and driven by a **length-dependent** (optimal with 40-mers) and **phosphorothioation dependent** interaction with exposed / uncomplexes hydrophobic surfaces of amphipathic alpha helices.

**REP 2055** is a 40-mer phosphorothioate oligodeoxyribonucleotide with the sequence (AC)20. **REP 2139** is an **RNA derivative** of REP 2055 in which each ribose is 2'O methylated and each cytosine base is 5-methylated.

The poly AC of REP 2139 sequence is designed to:
- eliminate intra / intermolecular interactions
- minimize secondary structure and off-target interactions
- avoid / minimize recognition by PRRs in order to render NAPs immunologically inert and devoided of immunostimulatory effect
- have and maintain the flexible B-form DNA structure required for optimal NAP activity




**REP 2055** 5' ACACACACACACACACACACACACACACACACACACAC 3'

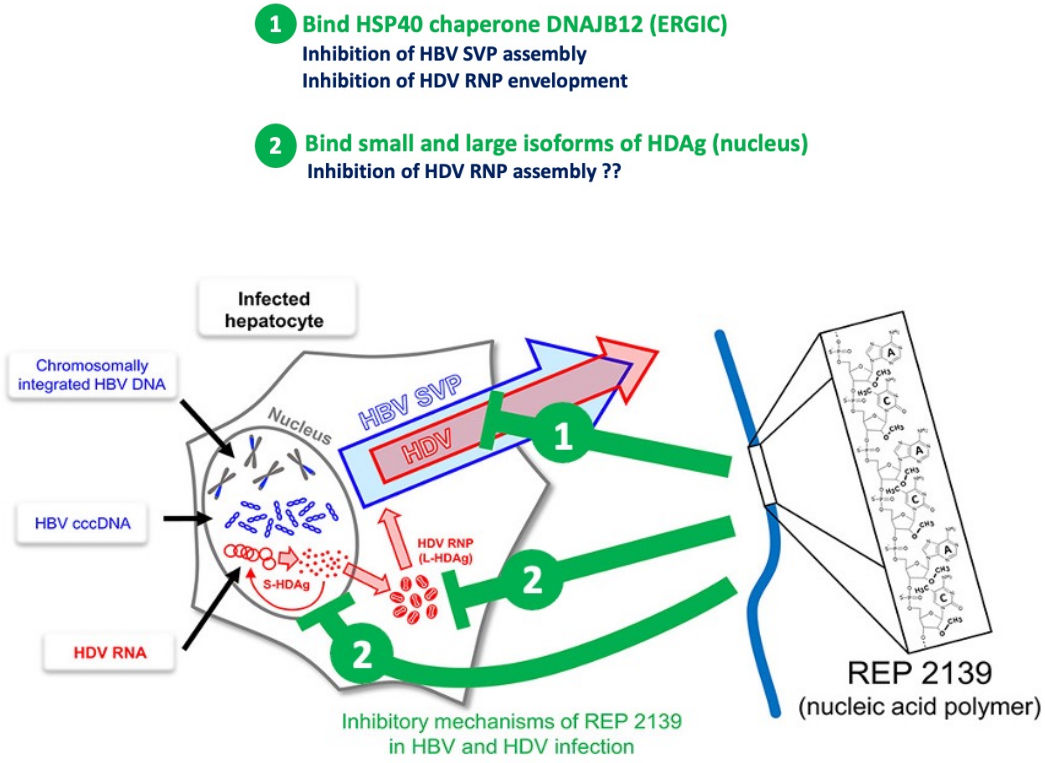
**REP 2139** 5' ACACACACACACACACACACACACACACACACACACAC 3'

**A = deoxyadenosine**
**C = 5-methylcytosine**
**_ = 2' O-methylated ribose**

# REP 2139 has multiple putative molecular mechanisms

REP 2139 Nucleic Acid Polymer (NAP)

- targets the **HSP40 chaperone DNAJB12** to **block HBV subviral particles assembly and secretion** (*Boulon et al, 2020*), an effect which also blocks HDV ribonucleoprotein (RNP) envelopment and HDV secretion from infected cell;
- **binds to S-HDAg and L-HDAg** in a fluorescence polarization (FP)-based, cell-free interaction assay (*Shamur et al, 2017*), raising the possibility of a direct effect in HDV RNP assembly



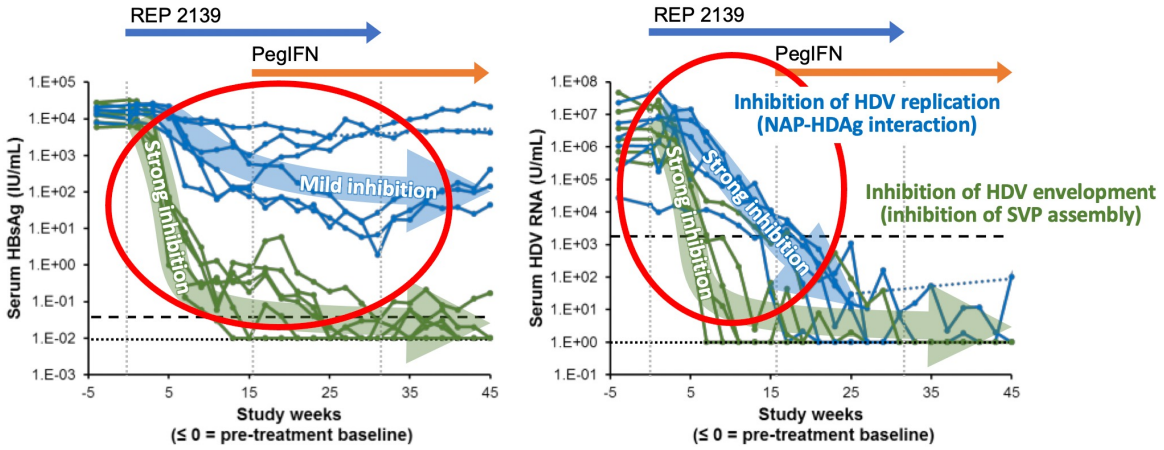
# REP2139 in HDV patients

A phase II study and current compassionate use of REP 2139 in HBV/HDV infection have shown a robust and early response toward HDV RNA as compared to HBsAg, suggesting a second direct acting antiviral mechanism.

Safety and efficacy of REP 2139 and pegylated interferon alfa-2a for treatment-naive patients with chronic hepatitis B virus and hepatitis D virus co-infection (REP 301 and REP 301-LTF): a non-randomised, open-label, phase 2 trial

*Lancet Gastroenterol Hepatol* 2017; 2: 877–89

*Michel Bazinet, Victor Pântea, Valentin Cebotarescu, Lilia Cojuhari, Pavlina Jimbei, Jeffrey Albrecht, Peter Schmid, Frédéric Le Gal, Emmanuel Gordien, Adalbert Krawczyk, Hrvoje Mijočević, Hadi Karimzadeh, Michael Roggendorf, Andrew Vaillant*




**The effect on HDV RNA before PEG-IFN is started and independent from the strength of the "HBsAg effect)**

# AIM

- To investigate the direct antiviral activity of HDV in relevant cell infection models *in vitro*.

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replicor

Andrew Vaillant

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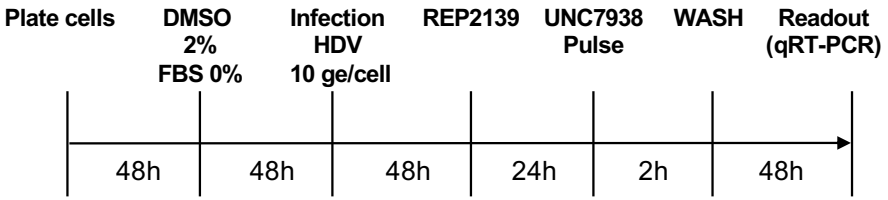
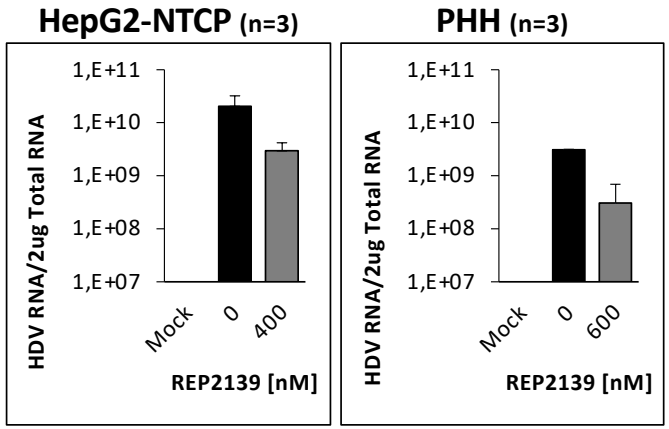
# METHODS

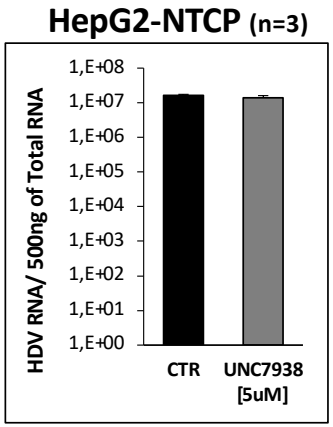
- REP 2139 endosomal release in 2D cultures *in vitro* was restored by UNC 7938 (*Blanchet, Antiviral Res 2019*).
- Clinical supply of REP 2139-Mg (lot FAB-22-0001) was used for dosing in HDV-infected (10 ge/cell) HepG2-NTCP cells, Huh7-END cells and primary human hepatocytes (PHH).
- Intracellular HDV viral genome levels were assessed by qRT-PCR and ddPCR
- 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*).
- Precision Cut Liver Cuts (PCLS) as a model for investigating REP-2139 MoA in a HDV-infected 3D liver model that includes hepatocytes and non-hepatocytic live cells (conserved cell polarization, cell-cell interactions)

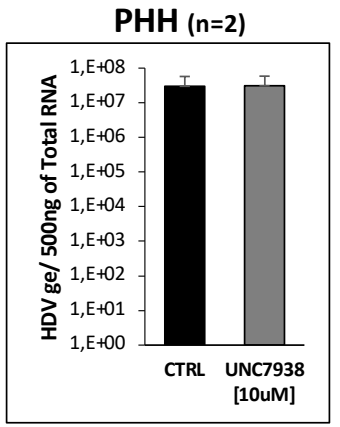


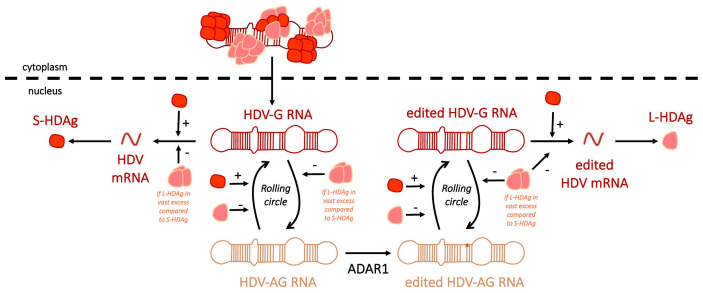






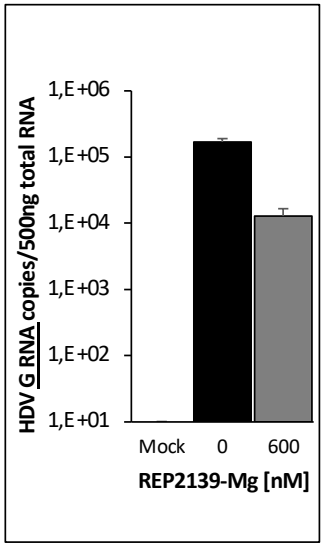

- **A single dose of REP 2139-Mg reduces intracellular HDV viral genome levels by ~1 log10 in HepG2-NTCP cells and PHH**
- **UNC7938 has no effect on HDV replication**

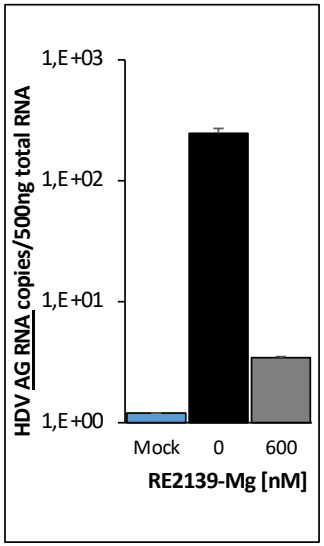



Lucifora J. and Delphin M. 2020

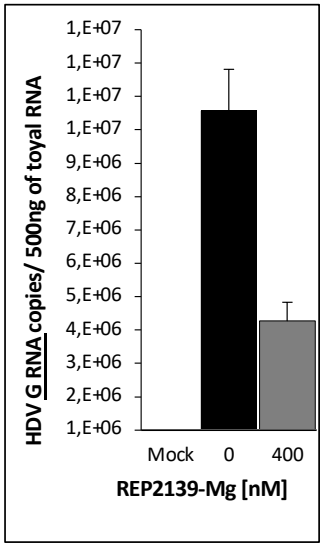
ddPCR

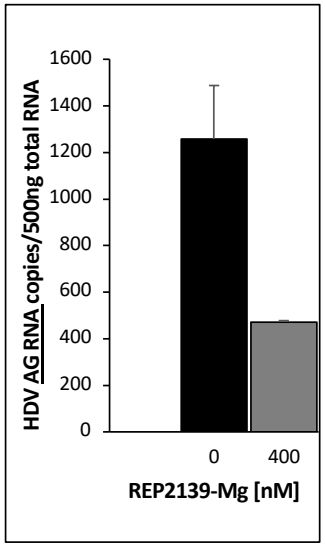
**PHH (n=3)**



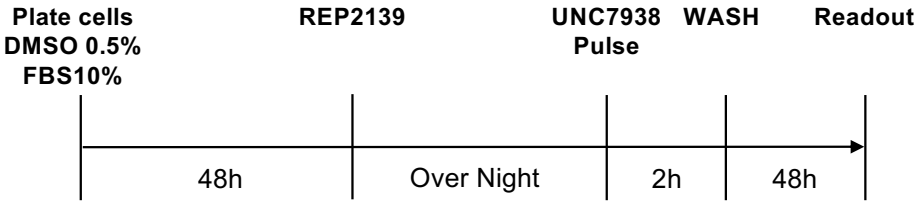




**HepG2-NTCP (n=3)**

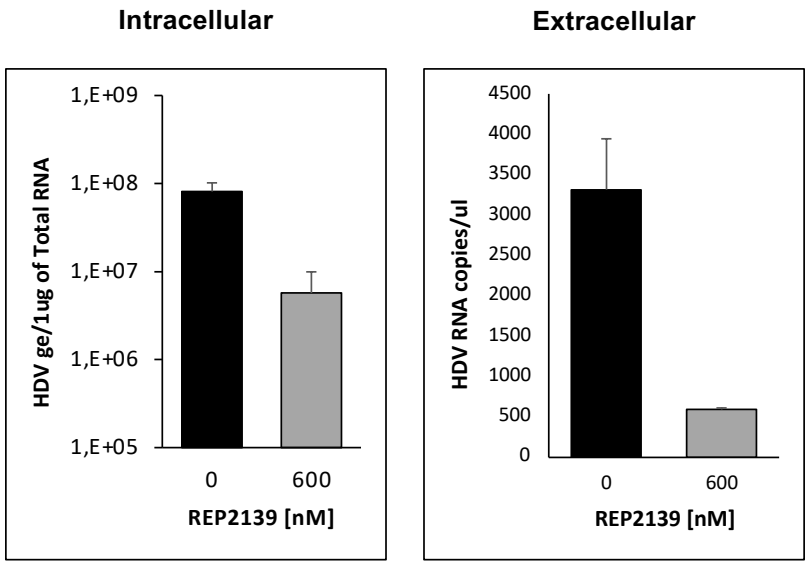


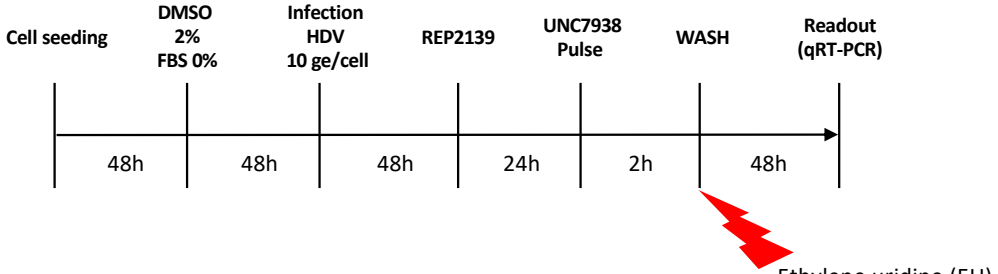

➢ REP 2139-Mg reduces both ***genomic*** and ***anti-genomic*** HDV RNA (***≃1Log***)




**HuH7-END (n=3)**

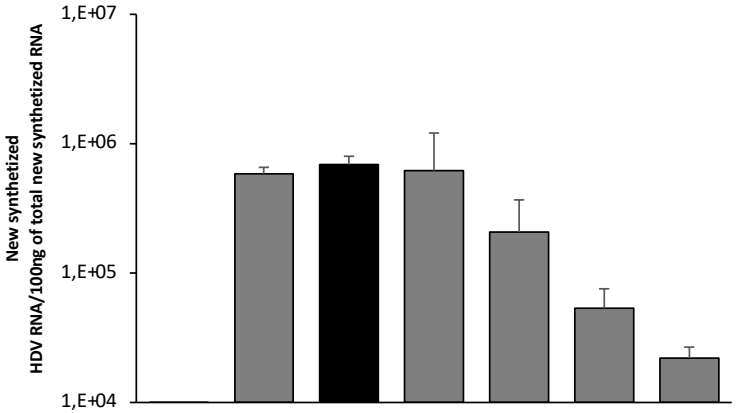



- A single dose of REP 2139-Mg reduces intracellular HDV viral genome and HDV virions secretion levels by ~1 $\log_{10}$ in the stable HDV replicating cells Huh7-END cell line that supports a complete HDV viral cycle and secretes HDV infectious virions




qPCR

**HepG2-NTCP (n=2)**

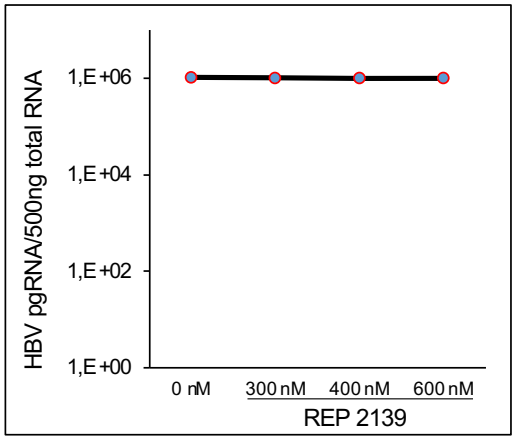



| HDV 10ge/cell | - | + | + | + | + | + | + |
|---|---|---|---|---|---|---|---|
| UNC7938 [5 uM] | - | - | + | + | + | + | + |
| REP2139-Mg [nM] | - | - | - | 200 | 400 | 600 | 800 |

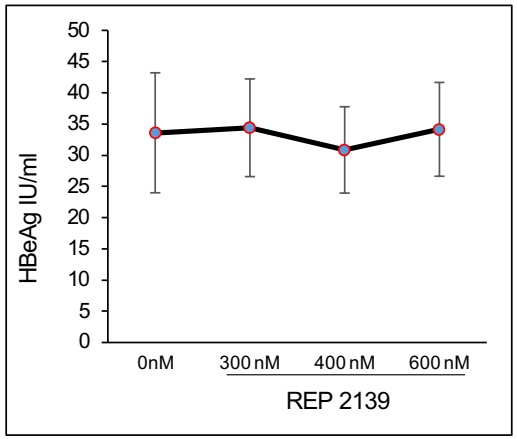
**≃1.5Log**

➢ ***De novo synthesis*** of HDV RNA is inhibited in presence of REP2139-Mg in a dose dependent manner

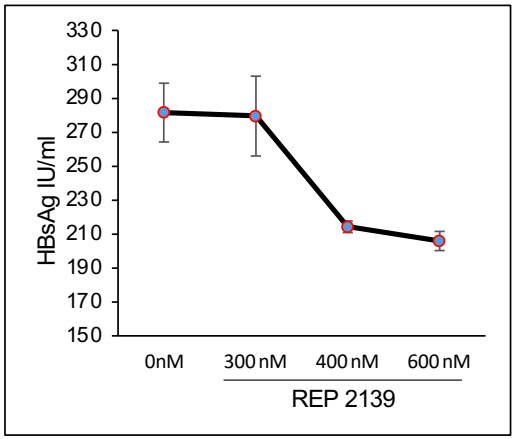
pgRNA (ddPCR)



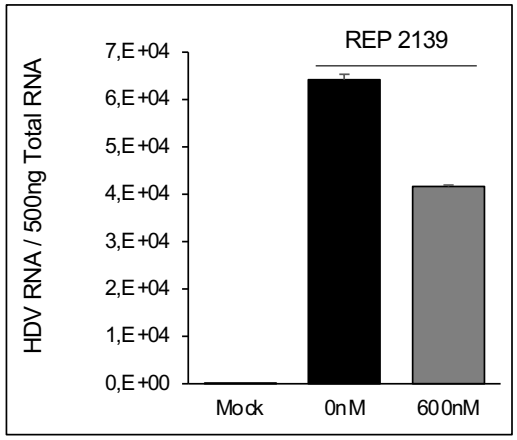

HBeAg (ELISA)




HBsAg (ELISA)




HDV RNA (ddPCR)




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

# Conclusion .1

- REP2139-Mg has a direct acting antiviral effect against HDV RNA replication

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***Limitations***

- the need for REP 2139 endosomal release in *in vitro* cell models by UNC 7938
- UNC 7938 toxicity, that limits the possibility
  - to increase REP 2139 and UNC 7938 dosing
  - to establish multiple dosing protocols
- short “therapeutic window” to achieve greater antiviral effect

# Conclusion .1

- REP2139-Mg has a direct acting antiviral effect against HDV RNA replication

Which is its molecular mechanism of REP 2139 direct antiviral effect on HDV?

Does the binding of REP2139 S-HDAg observed in a fluorescence polarization (FP)-based cell-free interaction assay interfere with the HDV RNP assembly?

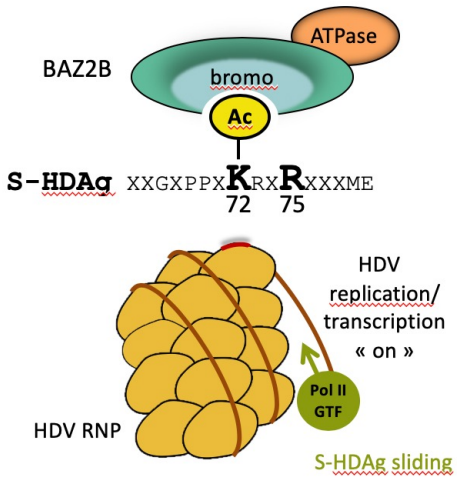
# HDV RNP (ribonucleoprotein) as an RNA pseudo-minichromosome

ARTICLE
https://doi.org/10.1038/s41467-020-14299-9 OPEN
nature COMMUNICATIONS

Hepatitis Delta Virus histone mimicry drives the recruitment of chromatin remodelers for viral RNA replication

Natali Abeywickrama-Samarakoon[1,9], Jean-Claude Cortay[1,9], Camille Sureau[2], Susanne Müller[3], Dulce Alfaiate[1,4,8], Francesca Guerrieri[1,5], Apirat Chaikuad[3], Martin Schröder[3], Philippe Merle[1,6], Massimo Levrero[1,5,6*] & Paul Dény[1,7*]

**S-HDAg possesses a histone-like function….**





**.... to hijack host Pol2 to an RNA pseudo-chromosomal template**

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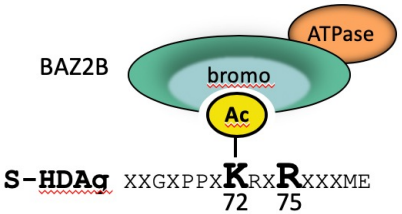
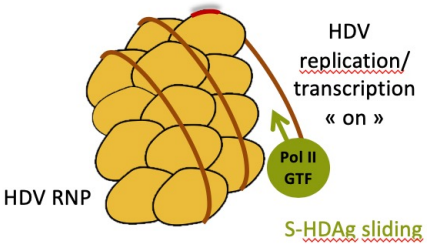
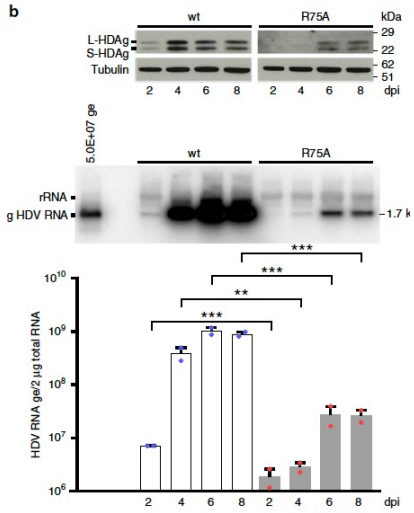
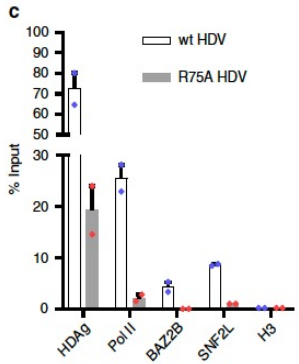


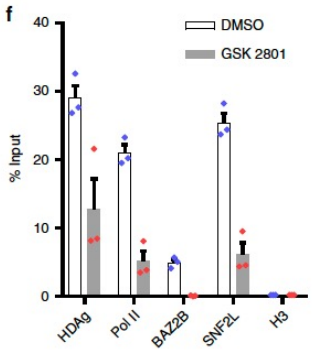

**.... to hijack host Pol2 to an RNA pseudo-chromosomal template**

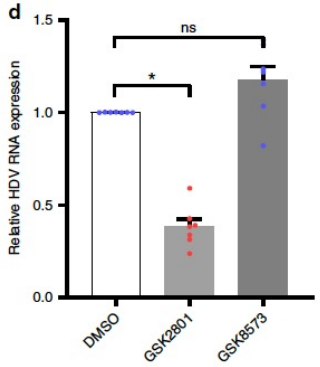

## HDV RNP (ribonucleoprotein) as a therapeutic target



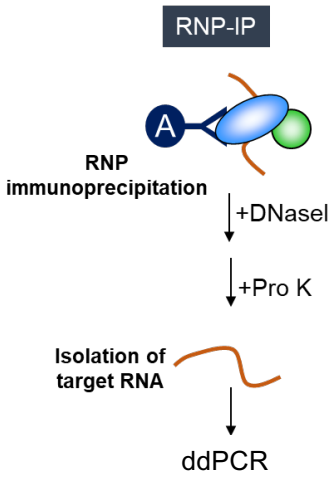



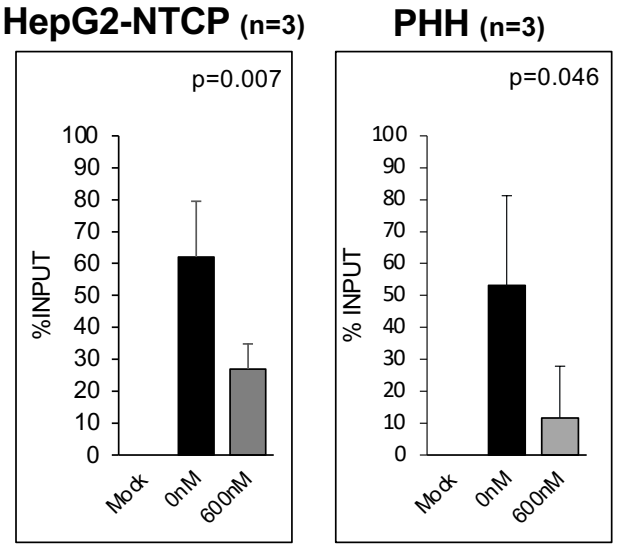

**The HDV R75A mutant virus, carrying a mutation that abrogates the interaction of S-HDAg with the BAZ2B BRD, shows a 2 Log reduction in HDV replication**



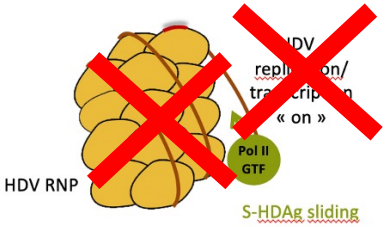

**The BRD inhibitor GSK2801 reduces BRF1/5 recruitment and inhibits HDV replication**

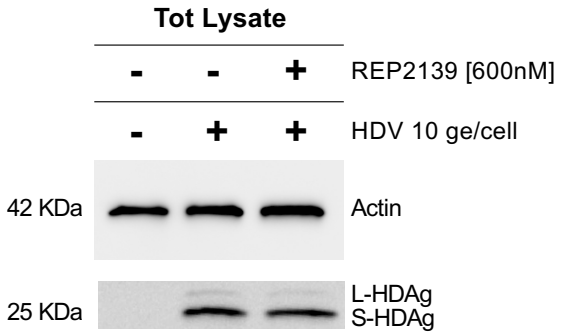




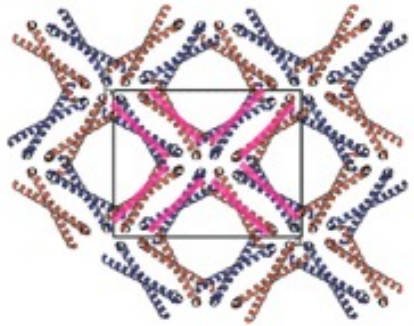




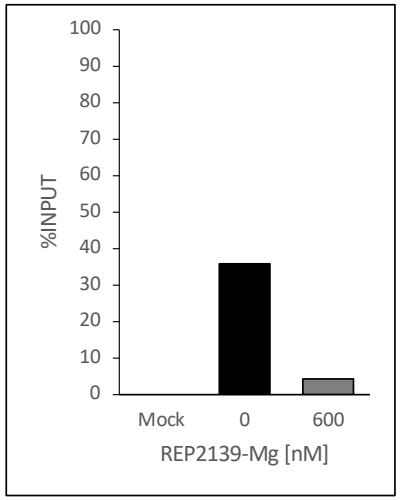

- A single REP 2139-Mg dose also reduced the intranuclear association of HDV RNA with HDAg by ~60% in HepG2-NTCP and by 80% in PHH, **without changing the HDAg protein levels**.

## Model for NAPs interaction with HDAg

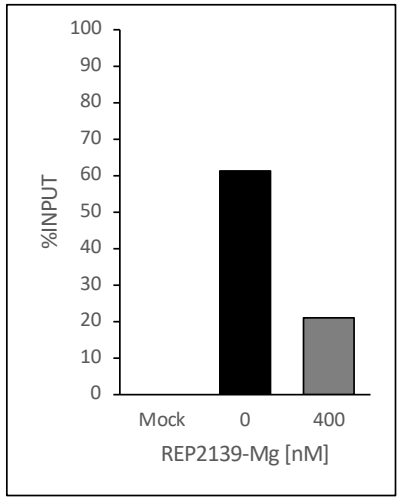


NAPs anneal to sHDAg amphipathic alpha helices and inhibits thiei functions

**HDVGP2**
**(Antibodies-online)**
**PHH**




**FD3A7**
**(Kerafast)**
**HepG2-NTCP**




- Similar anti-HDAg RIP results were obtained in both HDV infected PHHs and HepG2-NTCP cells **using additional anti-HDAg antibodies**
- This rules out the possibility that Rep2139-Mg could have masked the HDAg epitopes recognized by the CS antiboby

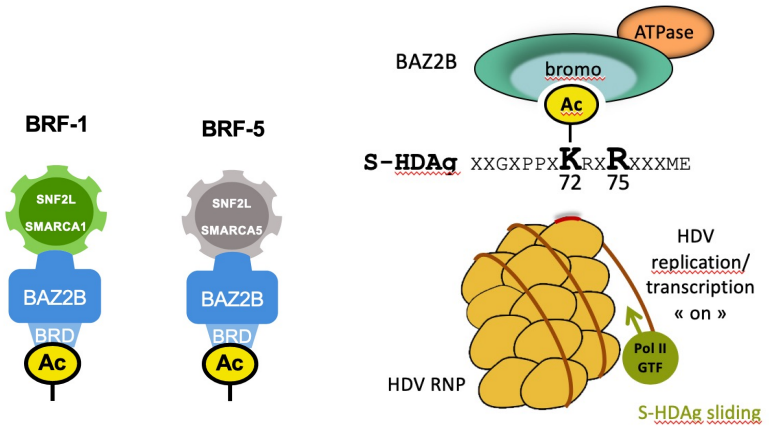
# Conclusion .2

- 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 may explain the more rapid decline of HDV RNA versus HBsAg in human studies.

# Conclusion .2

Ongoing experiments include

- experiments with “control” NAPs: REP 2184 (10 mer), REP 2147 (40 mer no PS) .....
- effect on HDV RNA half life
- impact on S-HDAg histone mimicry and HDV RNP interactome,
- impact on HDV infectivity



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