Modeling the post-entry activity of REP 2139 against HBV infection *in vitro:* an update.

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BACKGROUND & AIMS

- REP 2139 is a potent suppressor of serum HBsAg *in vivo* in the duck model and in proof of concept clinical trials[1-4].
- The post entry effect of REP 2139 appears to be essential for targeting HBsAg secretion and for clinical effect[5].
- Non-productive sequestration of phosphorothioate oligonucleotides in endosomes *in vitro* complicates modeling the effects of REP 2139[6,7].
- We present the continued study of the post-entry antiviral activity of REP 2139 *in vitro* observed with the endosomal release of REP 2139 following endocytosis in HepG2.2.15 cells, using the UNC 7938 compound[8].

MATERIALS & METHODS

- Endosomal release of REP 2139 in HepG2.2.15 cells was achieved by adding 10 to 15 µM UNC 7938 for 2 hours. Treatment paradigms are illustrated in figures below.
- Viability was monitored by MTS and total protein (BCA).
- HBsAg was monitored using Murex version 3 (Diasorin), HBeAg was monitored using HBeAg,ETI-EBK plus, N0140 (Diasorin).
- Cellular HBV RNA and PCSK9 mRNA was monitored by reverse transcription and Taqman qPCR following total RNA extraction.
- Fluorescence monitoring of CY3-labelled REP 2139 was performed on HepG2.2.15 cells cultured on glass coverslips, and fixed for 10 min in 4% formaldehyde. Nuclei were stained with DAPI.
- Reported HBsAg and HBeAg concentrations are normalized the control (UNC alone) and to total cellular protein, as measured by BCA.

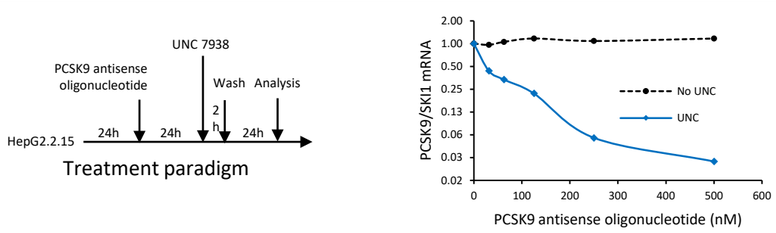

Figure 1. Endosomal release of PCKS9 antisense oligonucleotide with UNC 7938 is mandatory for its cytoplasmic antisense effect *in vitro* in HepG2.2.15 cells.

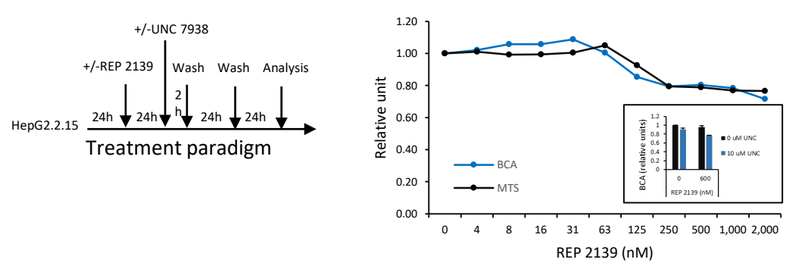

Figure 2. Effects on cell viability with REP 2139 and UNC. Inset: cell viability with REP 2139 in the presence or absence of UNC 7938.

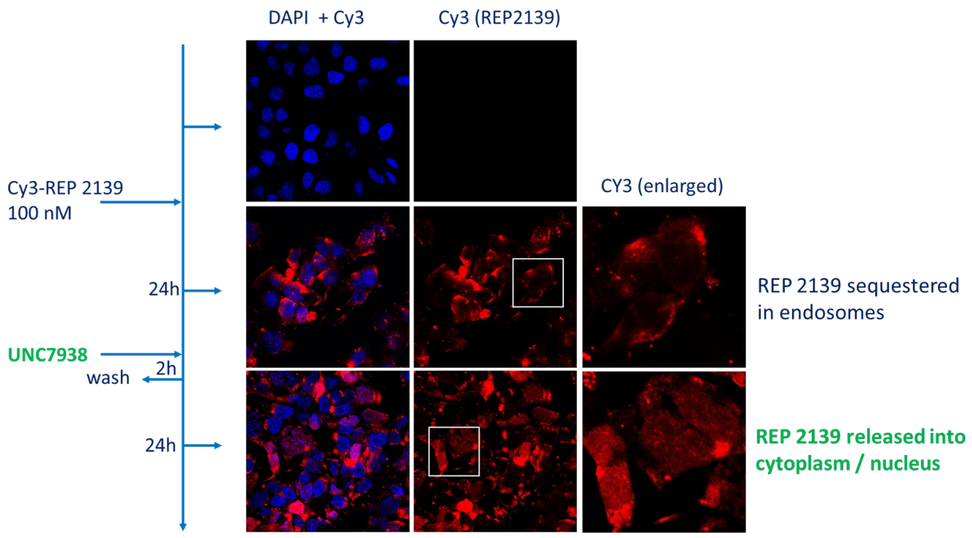

Figure 3: Effect of endosomal release of REP 2139 with UNC 7938 on cellular localization of CY3-REP 2139 in HepG2.2.15 cells (left) and release of HBsAg into the supernatant (right), following the treatment paradigm shown in Figure 2.

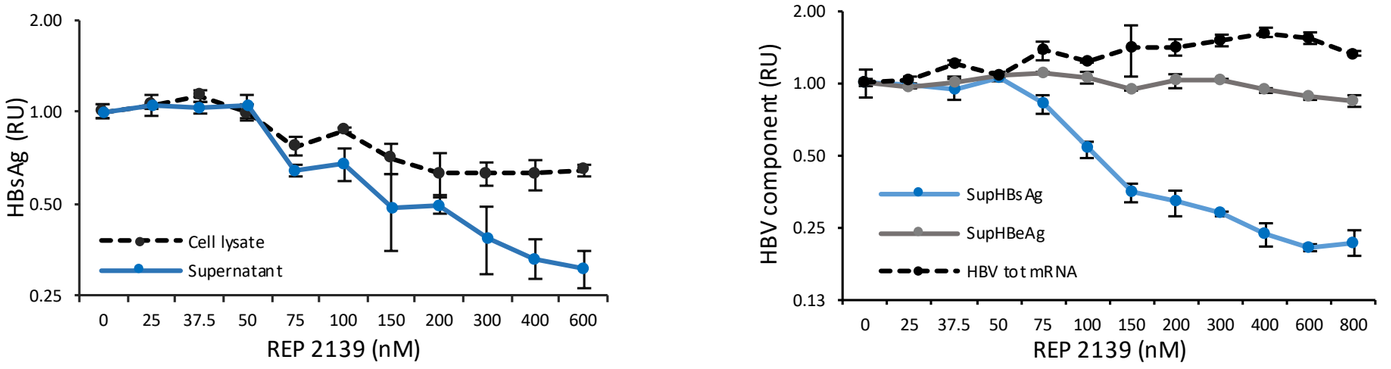

Figure 4. Effect of REP 2139 treatment on intracellular (cell lysate) and extracellular (supernatant) HBsAg (left) and on intracellular HBV total RNA, extracellular (supernatant) HBsAg and HBeAg following the treatment paradigm as shown in Figure 2.

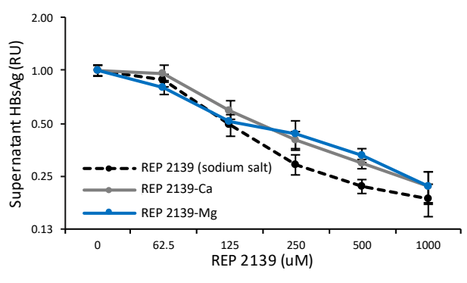

Figure 5. Formulations of REP 2139 used *in vivo* and in clinical studies have similar activity *in vitro*. Treatment paradigm is shown in Figure 2.

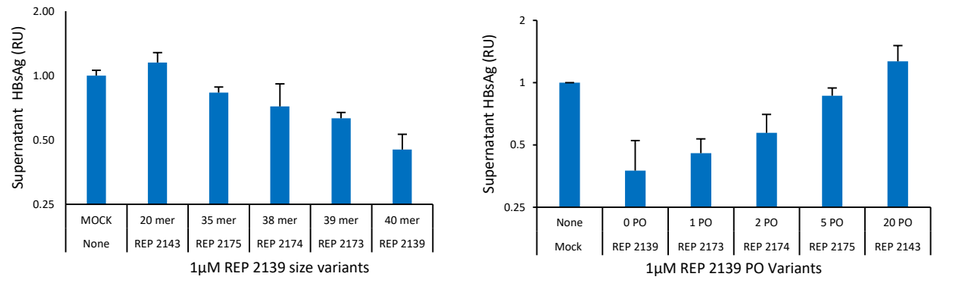

Figure 6. The antiviral effect of REP 2139 *in vitro* is size dependent (left) and is lost with increasing numbers of phosphodiester (PO) linkages (right). PO linkages cannot interact with hydrophobic surfaces[5]. Treatment paradigm is shown in Figure 2.

CONCLUSIONS

- **Endosomal release restores cytoplasmic functioning of oligonucleotides in HepG2.2.15 cells .**
- **Endosome release restores REP 2139 trafficking to the cytoplasm and nucleus.**
- **REP 2139 leads to a reduction in HBsAg concentration in the supernatant of HepG2.2.15 without its accumulation within the cell.**
- **REP 2139 effect is post-translational and appears selective for HBsAg.**
- **REP 2139 drug product formulations used in *in vivo* and clinical studies do not alter REP 2139 activity.**
- **The target interface involved in this effect is a host protein with a large exposed hydrophobic domain.**

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