Characterization of the antiviral effects of REP 2139 on the HBV lifecycle *in vitro*

Boulon R[1], Blanchet M[1,2], Lemasson M[3], Angelo L[1], Vaillant A[2], and Labonté P[1].

1.Institut National de la Recherche Scientifique – Institut Armand Frappier, Laval, Canada, H7V 1B7.
2.Replicor Inc. Montréal, Canada, H4P 2R2.
3.Institut National de la Transfusion Sanguine, CNRS-INSERM U1134, Paris, France, 75739.

INTRODUCTION

- In HBV infection, HBsAg plays an important role in allowing HBV to chronically persist by interfering with immune function[1].
- Subviral particles are the primary source of HBsAg, whose assembly and secretion is not dependent on cccDNA and is independent from virions[2] (Figure 1).
- In both pre-clinical and clinical studies, the nucleic acid polymer REP 2139 is accompanied by clearance of HBsAg from the blood[3-6]. When REP 2139 is combined with pegylated interferon and tenofovir disoproxil fumarate, high rates of functional control (inactive chronic HBV) or functional cure are achieved.
- REP 2139 has been shown to block assembly and secretion of SVP *in vitro* in HepG2.2.15 cells, accompanied by reduction in intracellular HBsAg[7].
- The other effects of REP 2139 on other aspects of the viral life cycle have not yet been established and are currently being examined *in vitro* using the UNC 7938-HepG2.2.15 model.

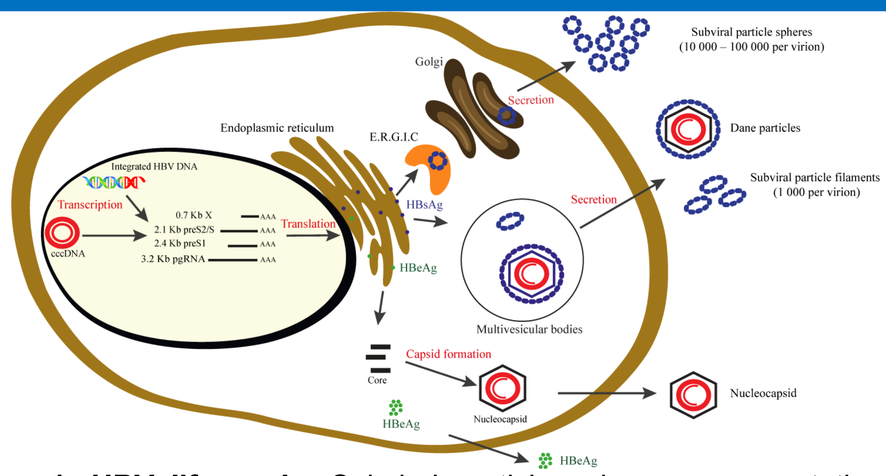

Figure 1. HBV life cycle. Subviral particle spheres represent the bulk of circulating HBsAg.

MATERIALS & METHODS

- Endosomal release of REP 2139 in HepG2.2.15 cells was achieved by adding 15 µM UNC 7938[8] for 2 hours. Treatment paradigms are illustrated in figure 2.
- Viability was monitored by total protein (BCA).
- Cellular HBV RNA and SKI1-S1P mRNA was monitored by reverse transcription and qPCR following total RNA extraction and normalization by optical density (OD_{260}). HBV RNA was also analyzed by Northern-blot and quantified by phospho-Imager.
- Cellular HBV DNA was monitored by qPCR following total DNA extraction after normalization by optical density (OD_{260}).
- HBsAg was monitored using Murex version 3 (Diasorin), HBeAg was monitored using ETI-EBK plus, N0140 (Diasorin).
- Dane particle was monitored by immunoprecipitation using anti-preS1 antibody (Hep B preS1 Antibody (AP1): sc-57761, Santacruz) followed by DNA extraction and quantification by qPCR.
- Reported data are normalized to the control (UNC alone).

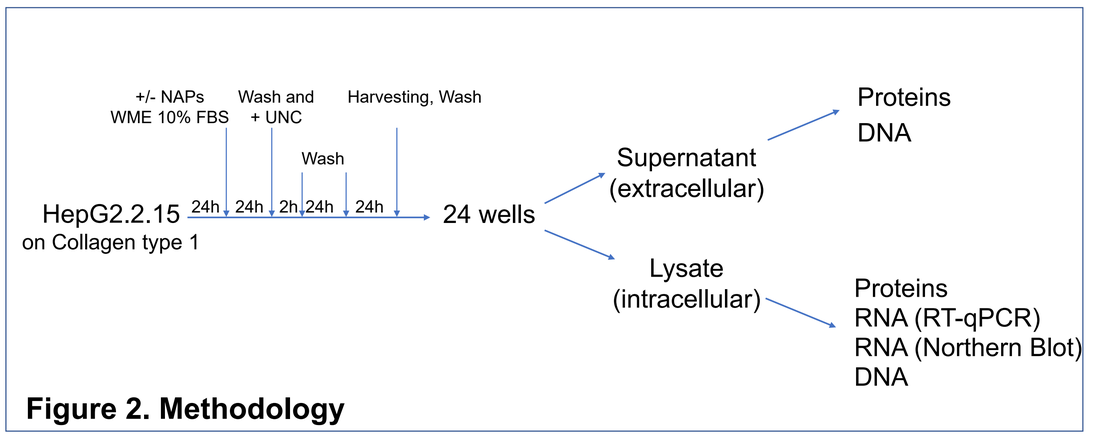

Figure 2. Methodology

RESULTS

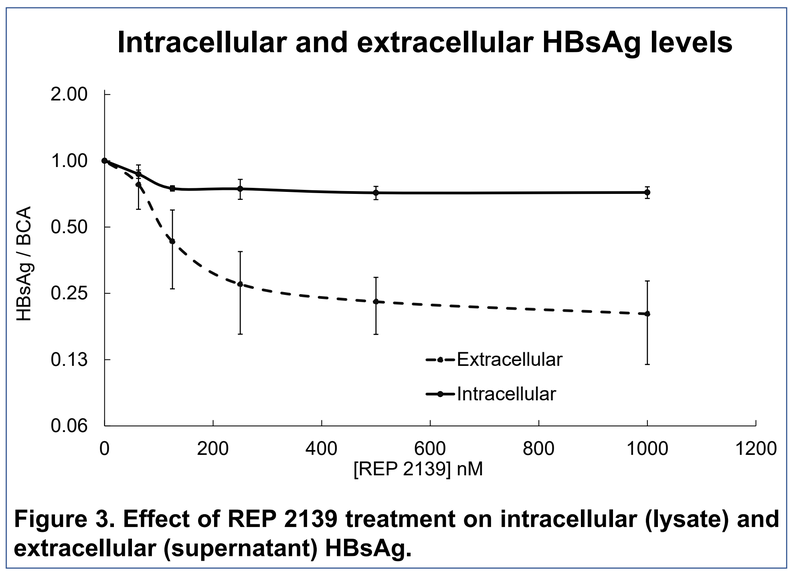

Figure 3. Effect of REP 2139 treatment on intracellular (lysate) and extracellular (supernatant) HBsAg.

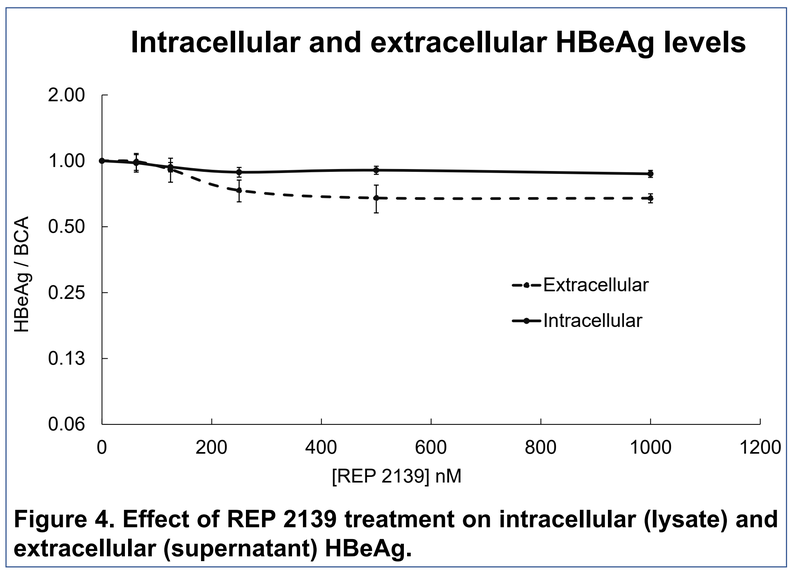

Figure 4. Effect of REP 2139 treatment on intracellular (lysate) and extracellular (supernatant) HBeAg.

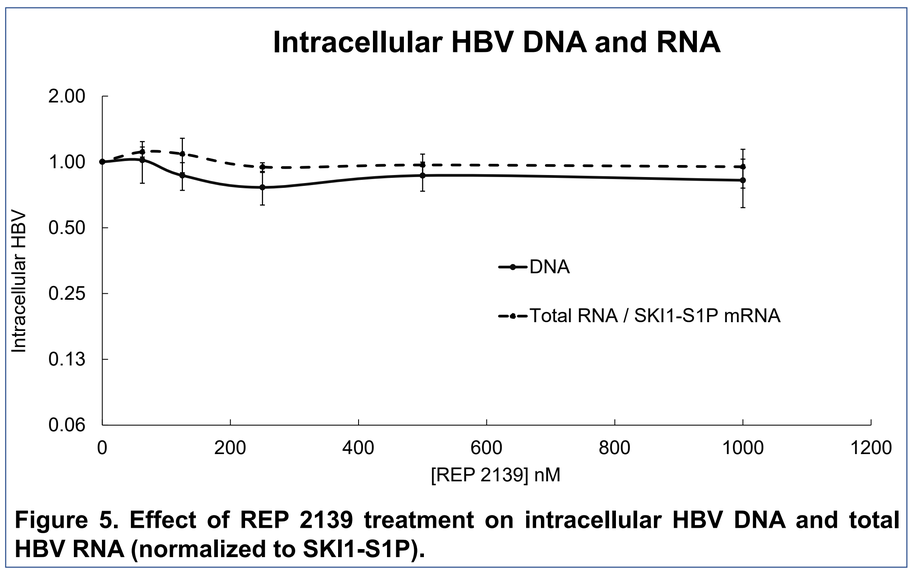

Figure 5. Effect of REP 2139 treatment on intracellular HBV DNA and total HBV RNA (normalized to SKI1-S1P).

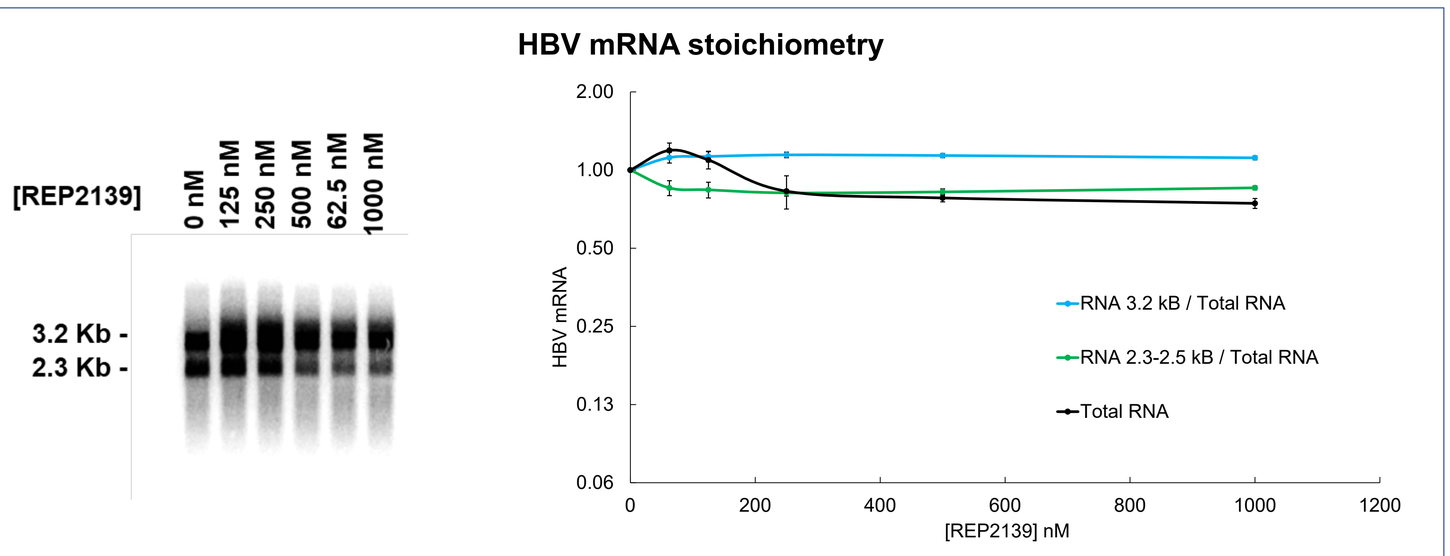

Figure 6. Effect of REP 2139 treatment on intracellular (cell lysate) HBV mRNA 3.2 Kb and 2.3 Kb.

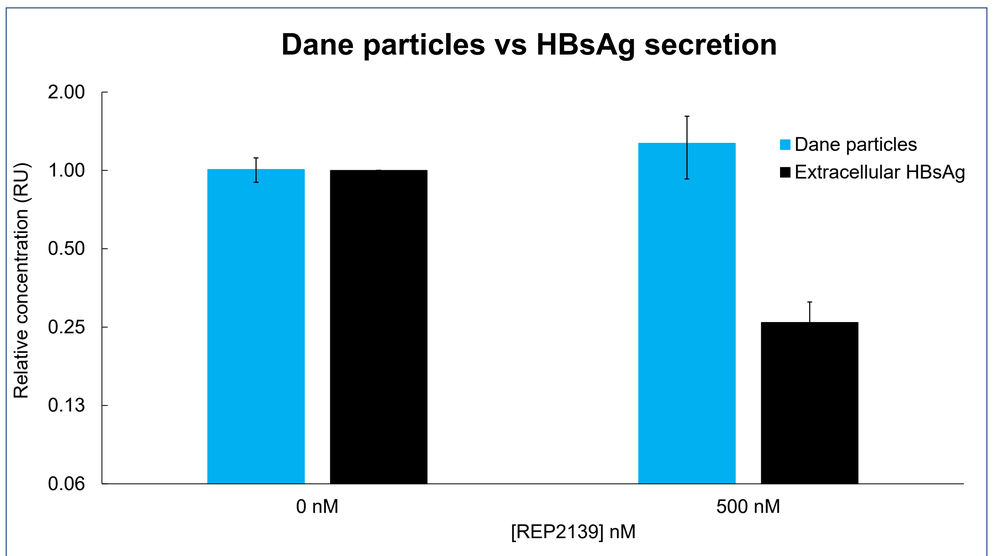

Figure 7. Effect of REP 2139 treatment on the secretion of Dane particles and HBsAg (both are normalized to BCA).

CONCLUSIONS

- In ongoing studies in this model, declines in extracellular and intracellular HBsAg were similar to those previously published, with IC_{50} in the 100nM range[7].
- Minor reduction in extracellular HBeAg was not accompanied by declines in intracellular HBeAg.
- Slight declines in intracellular HBV DNA appeared to follow initial mild increases in HBV RNA with REP 2139 dose escalation up to 250 nM but both reverted to baseline levels at higher doses.
- Mild increases in HBV total RNA, quantified by Northern-Blot, appeared to be due to accumulation of 3.5 kB HBV RNA species.
- Secretion of HBV Dane particles was not affected by REP 2139 at 500 nM.
- REP 2139 inhibits secretion of HBsAg without altering transcription and translation of viral components or secretion of HBeAg or Dane particles.
- Analysis of changes in HBsAg isoform stoichiometry are currently underway to complete the characterization of the antiviral effects of REP 2139.
- Effects are consistent with selective interference of REP 2139 with assembly/secretion of SVP.

REFERENCES

1. Kondo et al. ISRN Gastroenterol. **2013**; 2013: 935295.
2. Shao et al. W J Gastroenterol. **2007** Apr 14; 13(14): 2104–2107.
3. Vaillant. Antivir. Res. **2016**; 133, 32-40.
4. Bazinet et al. J Hepatol. **2018**; 68: S517.
5. Bazinet et al. Lancet Gastro Hepatol. **2017**; 2: 877-889.
6. Vaillant. ACS Inf Dis. **2019**; 10: 675-687.
7. Blanchet et al., Antiviral Res. **2019**; 165: 97-105.
8. Yang et al., Nuc Acid Res. 2015;43:1987-1996.

Contact information:

patrick.labonte@iaf.inrs.ca
availlant@replicor.com