Post-entry antiviral effects of nucleic acid polymers against HBV infection in vitro

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INTRODUCTION
• Nucleic acid polymers (NAPs) are phosphorothioated oligonucleotides that have demonstrated a great potential to inhibit HBV and HDV infection.
• NAPs are potent suppressors of serum HBsAg in vivo in the duck model and in proof of concept clinical trials.
• Certain NAPs exert antiviral properties at the entry step of HBV in vitro. However, REP 2139, which is clinically active, has no entry activity.
• The post-entry effect appears to be essential for targeting HBV infection and for clinical effect.
• NAPs, as for other phosphorothioated oligonucleotides (i.e. antisense), do not exert significant activity within the cell when provided without a delivery system. This has made the demonstration of a post-entry effect of NAPs in previous in vivo studies difficult.

AIM
To develop an in vitro model that replicates the post-entry antiviral effect of NAPs in HBV infection in vivo and in the clinic to further characterize the molecular targets involved in the inhibition of HBVAg secretion by NAPs.

METHODS
• HepG2.2.15 cells stably expressing HBV were used in this study.
• Cell culture, treatment and harvesting were conducted as described in each result section.
• Electroporation (Es) parameters (apparatus name) were as follows: 4 pulses of 90 μs at 250V with a 1.1 s interval.
• EUSA kits used in this study are as follows: HBsAg, Murex version 3 (Dacron); hAlbunin, Alcem ab70887.
• PCSK9 mRNA was quantified by reverse transcription using iScript kit. Probes (FAM-tcaacgaccgaccttga-dabcyl-MBG).
• HepG2.2.15 HBV virions from the supernatant were immunoprecipitated using an protein A-Sepharose 4B (Amersham Biosciences). Viral DNA was extracted using QIAamp DNA Mini kit (Qiagen) and quantified by Taqman qPCR using (5′-GACAGAGATGCTGGATGGTTGGTTTTCTGC-3′) and (5′-GACAGAGATGCTGGATGGTTGGTTTTCTGC-3′) primers.
• HBV virion DNA was monitored by reverse transcription and Taqman qPCR as described above.
• Results were normalized to the concentration of total cellular protein in cell lysates, as measured by BCA.
• Cells viability was measured using the CellTiter96 Aqueous One Solution Cell proliferation assay (Promega) and is expressed relative to the normal saline electroporation.

NAPs used in the study

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<th>Concentration in supernatant (RU)</th>
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CONCLUSIONS
• Electroporation of NAPs in HepG2.2.15 cells can reproduce the post-entry antiviral effects of NAPs observed in vivo and in patients.
• REP 2055 selectively inhibits HBsAg secretion without affecting intracellular HBsAg levels, virion maturation and egress, or albumin secretion.
• These effects suggest that NAPs act by a post-translational mechanism that selectively interferes with assembly and or egress of SVP.
• In this model, REP 2139 displays a broader activity, leading to decreased concentrations of HBsAg, HBV virions and albumin in the supernatant without major alteration of cell viability.
• The REP 2139 effects observed in vitro in this model differs from its observed in vivo and clinical activity, where REP 2139 exerts a selective effect on SVP secretion similar to REP 2055.

ACKNOWLEDGEMENTS
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REFERENCES

DISCLOSURES
MB and AV are employees of Replicor Inc. AV is a shareholder in Replicor Inc. PL has nothing to disclose.

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