

THE INTERNATIONAL LIVER CONGRESS™





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 HBsAg secretion 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 vitro 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 HBsAg 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 panel.
- Electroporation (Ep) parameters (apparatus name) were as follows: 4 pulses of 99 uS at 820V with a 1.1 s interval.
- ELISA kits used in this study are as follows: HBsAg, Murex version 3 (Diasorin); hAlbumin, Abcam ab179887.
- PCSK9 mRNA was quantified by reverse transcription using iScript select cDNA Synthesis kit (Biorad) and qPCR using Ssofast Evagreen supermix (Biorad) with the following primers : ggatacctcaccaagatcct, R-caacttcaaggccagctc.
- HBV virions from the supernatant were immunoprecipitated using an anti-preS1 antibody (Santa Cruz, sc-57761) and protein A/G agarose beads (Santa cruz, sc-2003). Viral DNA was extracted using QIAamp DNA mini kit (Qiagen) and quantified by Taqman qPCR using Ssoadvanced probe universal supermix (Biorad). Primers used were as follows: F-acgtcctttgtttacgtcccg, R-ccaactcctcccagtctttaaac Probe sequence was as follows: FAM-tcaacgaccgaccttga-dabcyl-MBG.
- Cellular HBV RNA 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.
- Cell viability was measured using the CellTiter 96 Aqueous One Solution Cell proliferation assay (Promega) and is expressed relative to the normal saline electroporation.

NAPs used in the study

Name	NAPs Sequence (5' – 3')	Chemical Modifications		
		PS	2'OMe	5-MeC
REP 2055	ACACACACACACACACACACACACACACACACACACAC	+		
REP 2139	ACACACACACACACACACACACACACACACACACACAC	+	+	+
REP 2147	ACACACACACACACACACACACACACACACACACACAC		+	+

PS = phosphorothioate, 2'Ome = 2' O methylation of ribose, 5-MeC = 5' methylation of cytosine base

R	ES	SL
A		
В		
_	1. A c 5. (A) ct.	
A		
В	Viability: 2 () 1	100
	1 Concentration (RU) 0.25 0.125	0
	4. Eff tmen h.	
A		
В	Acentration in supernatant (RU)	4 2 1
	icentration in supern	1 0.5

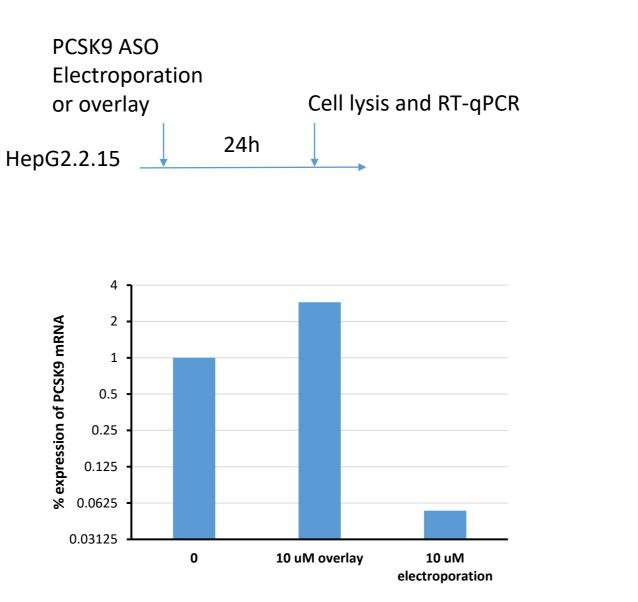


Post-entry antiviral effects of nucleic acid polymers against HBV infection in vitro

M. Blanchet ¹, <u>A. Vaillant¹</u>, P. Labonté²

1 Replicor Inc., Montréal, Canada, H4P 2R2 2 INRS-Institut Armand-Frappier, Laval, Canada, H7V 1B7

JLTS



very system is required for antisense activity in HepG2.2.15 tment paradigm. (B) Effect of electroporation on antisense

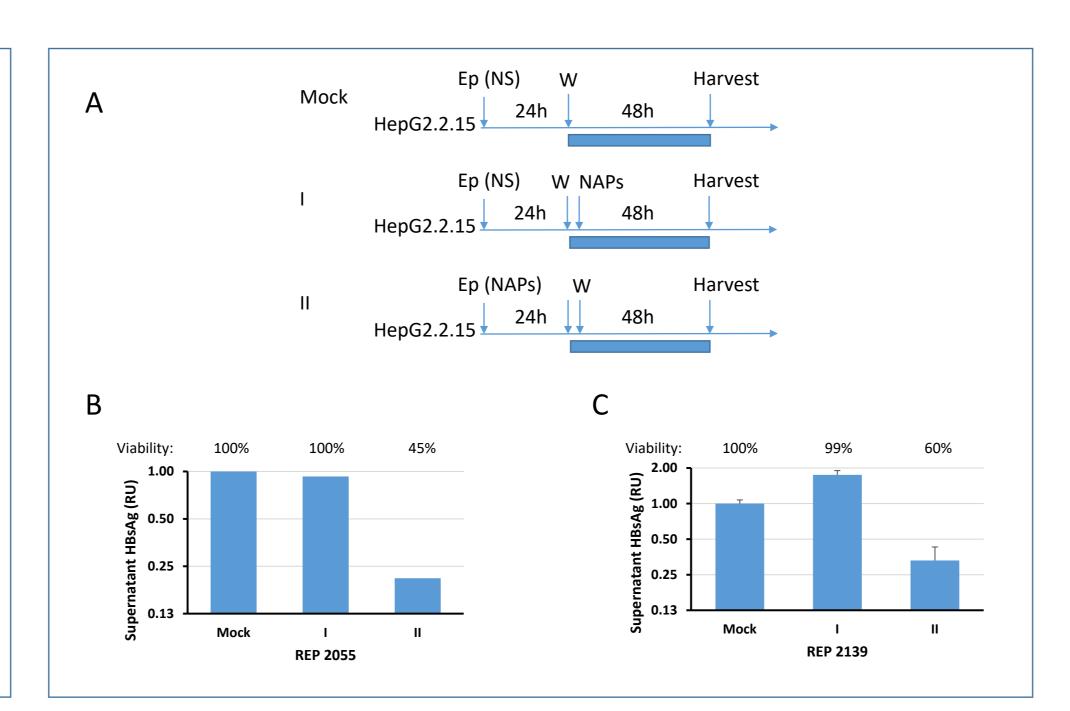
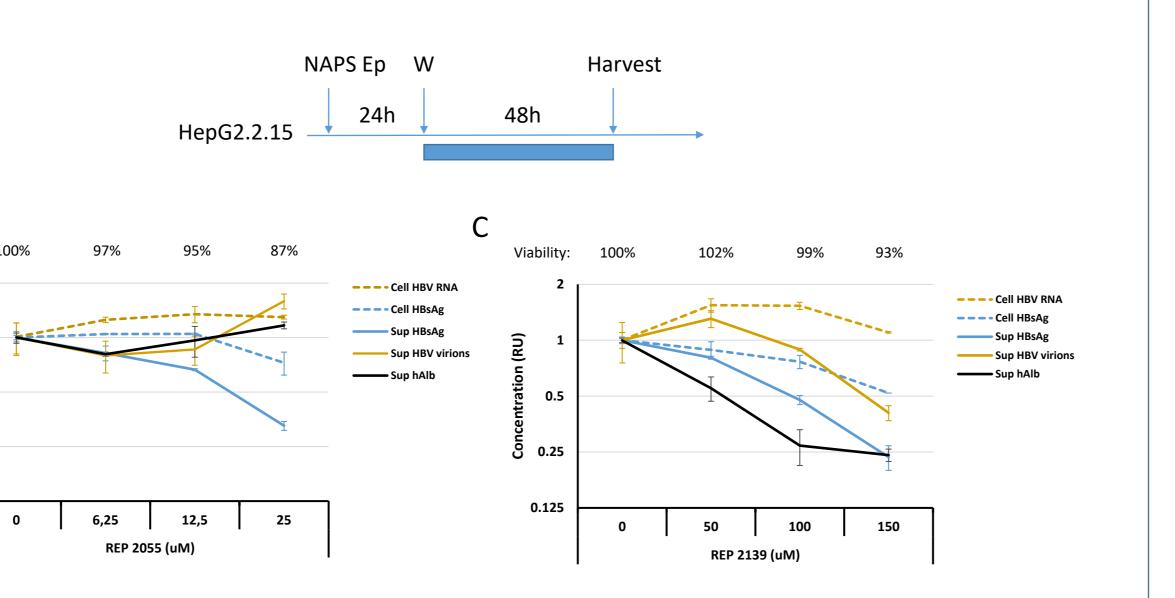


Fig. 2. Electroporation is required for NAP antiviral activity in HepG2.2.15 cells. A) treatment paradigm. HBsAg reduction in the supernatant with 25 uM REP 2055 (B) or 150 uM REP 2139 (C). Ep, electroporation; W, wash; NS, normal saline.





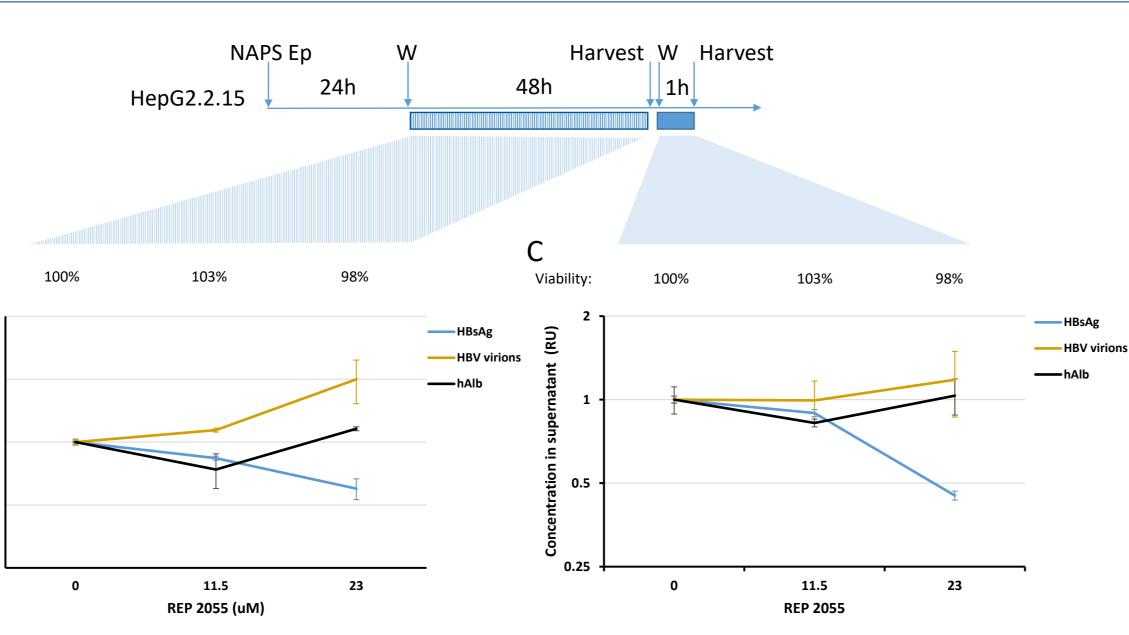
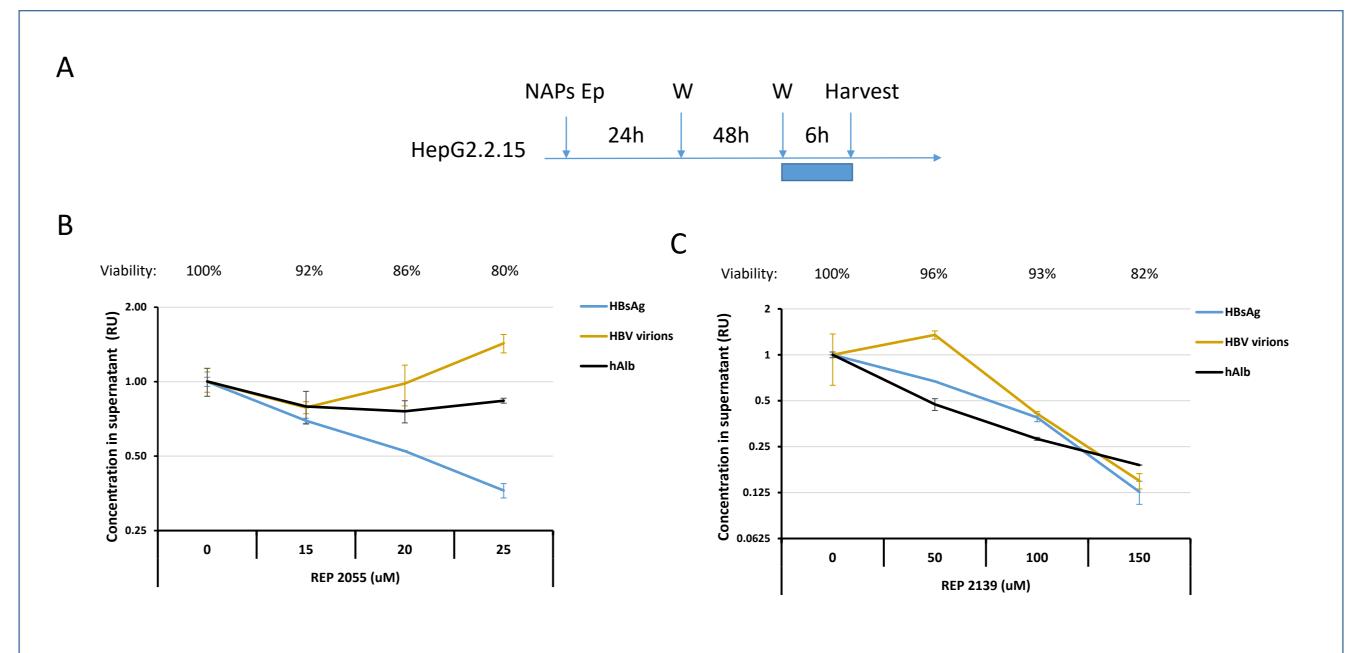
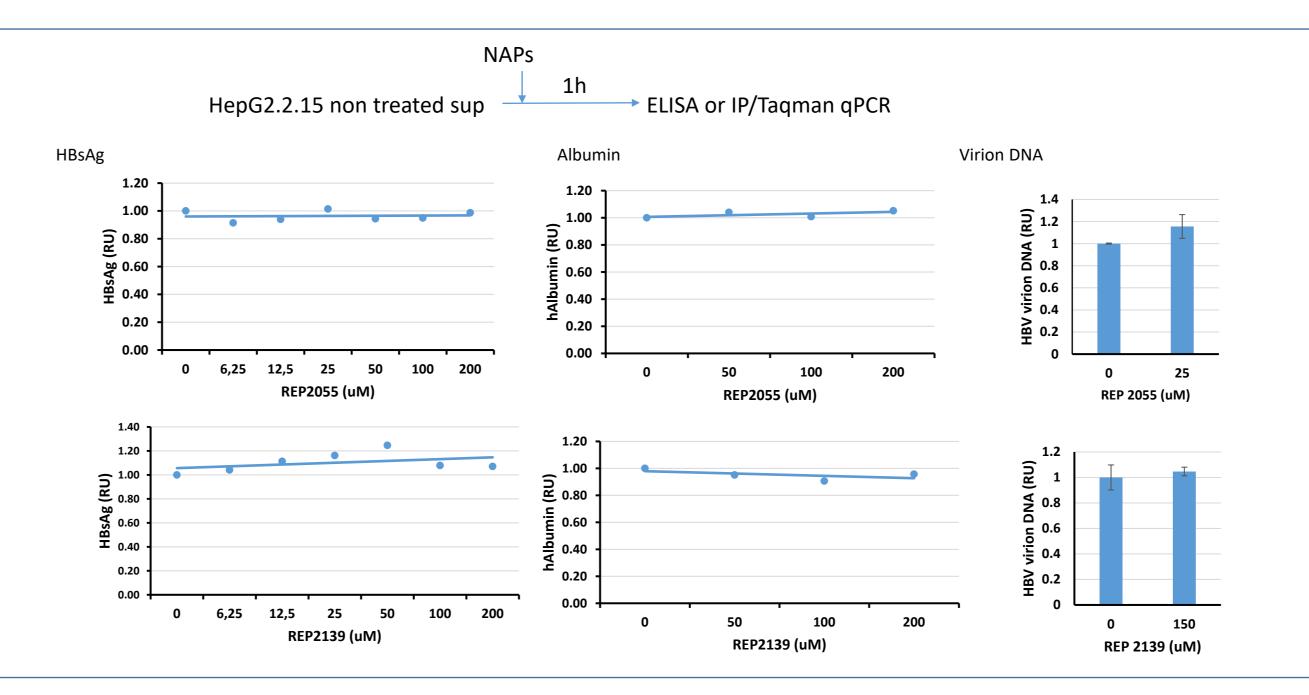


Fig. 6. Effect of HepG2.2.15 electroporation with REP 2055 on HBV lifecycle and on albumin secretion. (A) treatment paradigm. (B) and (C) results with accumulation of 48 h and 1h, respectively. Ep, electroporation; W, wash.



wash



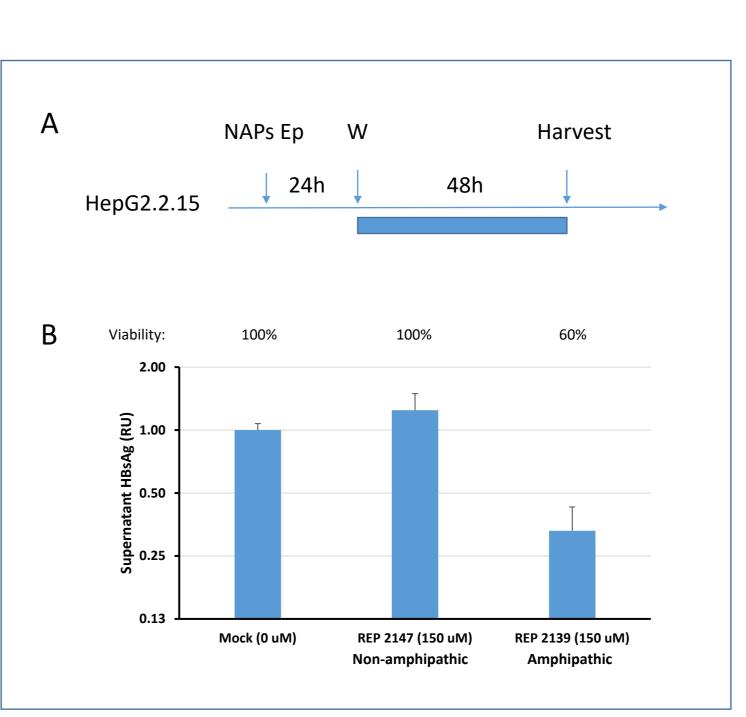


Fig. 3. Phosphorothioation of NAPs (amphipathicity) is mandatory for antiviral activity. Ep, electroporation; W, wash.



Fig. 7. Spiking experiments. NAPs do not interfere with ELISA quantification of HBsAg and Albumin, or with IP-extraction-Tagman guantification of virion-derived DNA



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

This work was supported Replicor Inc.

REFERENCES

- Noordeen, F et al. (2013). Antimicrob. Agents Chemother. 57: 5299-5306.
- Noordeen, F. et al. (2015). PLOS ONE 10: e0140909Al-Mahtab et al., 2016
- Al-Mahtab, M. et al. (2016). PLOS ONE 11: e0156667
- Quinet, J., et al. (2016). J. Hepatol. 64: S385
- Bazinet, M., et al. (2016). Hepatol. 64: 1122A
- Vaillant, A. (2016). Antiviral Res. 133: 32-40

DISCLOSURES

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

CONTACT INFORMATION

Andrew Vaillant: availlant@replicor.com

Patrick Labonté: patrick.labonte@iaf.inrs.ca