

Effects of nucleic acid polymers on hepatitis B virus entry in HepaRG cells and primary human hepatocytes

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BACKGROUND & AIMS

- Nucleic acid polymers (NAPs) are phosphorothioated oligonucleotides which exhibit a sequence independent, broad spectrum antiviral activity (reviewed in Vaillant, 2016).
- NAPs inhibit duck hepatitis B virus (DHBV) infection *in vivo* (Noordeen et al., 2013a, 2015) and HBV infection in proof of concept clinical trials (Al-Mahtab et al., 2016).
- NAPs have been previously shown to have entry and post-entry antiviral activities (Noordeen et al., 2013b) *in vitro* and act *in vivo* to block the release of HBsAg from infected hepatocytes (Noordeen et al., 2015).
- This study reports the *in vitro* antiviral effects of various NAPs at the entry step of HBV using HepaRG and primary human hepatocytes (PHH) models.

MATERIAL & METHODS

- Differentiated HepaRG cells and PHH were inoculated with HBV and treated with NAPs (see table 1) as depicted in diagrams in the results section.
- HBsAg and HBeAg in the supernatant (sup) were determined using the Elecsys HBsAg ELISA assay and the Autobio diagnostics immunoassay, respectively. Intracellular (cell) HBV RNA was measured by RT-qPCR
- Statistical analysis: unpaired, 2-tailed t-tests were used for result presented in F (lower panel) and G sections. Other results were analysed using one-way ANOVA.

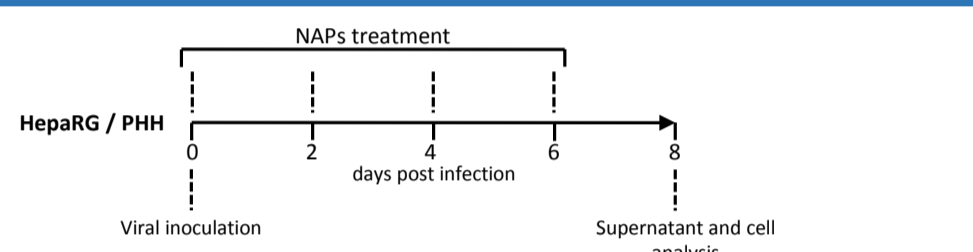
Table 1. Description of NAPs used.

Name	Sequence 5' - 3'	Length	Modifications			Chemistry
			PS	2'OMe (RNA)	5'MeC	
REP 2006	(N) ₄₀ (degenerate)	40	+			amphipathic (contains CpG)
REP 2107	(N) ₄₀ (degenerate)	40	+	+		amphipathic (contains CpG)
REP 2055	(AC) ₂₀	40	+			amphipathic
REP 2139	(AC) ₂₀	40	+	+	+	amphipathic
REP 2165	(AC) ₂₀	40	+	++	+	amphipathic (REP 2139 variant designed to degrade more rapidly)
REP 2172	(AC) ₂₀	40		+		non amphipathic (polyanionic) variant of REP 2055
REP 2147	(AC) ₂₀	40		+	+	non amphipathic (polyanionic) variant of REP 2139
REP 2149	(AC) ₃₀	60	+			REP 2055 size variants
REP 2150	(AC) ₁₅	30	+			
REP 2151	(AC) ₁₀	20	+			
REP 2152	(AC) ₅	10	+			
REP 2031	(C) ₄₀	40	+			
REP 2138	(C) ₄₀	40		+		non-amphipathic (polyanionic) variant of REP 2031

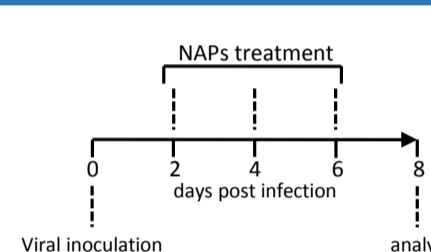
PS = phosphorothioation of phosphodiester linkage (increases amphipathicity)
 2'OMe = O-linked methylation at 2' position in ribose (increased stability to nuclease attack and reduced TLR reactivity)
 5'MeC = methylation of 5' position in cytidine base (reduced TLR reactivity)
 * Positions 11, 21 and 31 have 2'OH ribose
Active against HBV *in vitro* (in this study)
Active against HBV and HDV in clinical trials but inactive *in vitro* (in this study)
Active against HBV and/or HDV in clinical trials

RESULTS

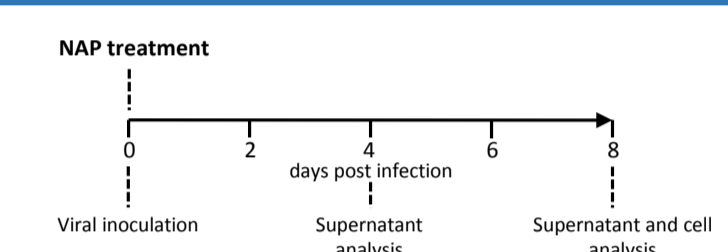
Co + post-treatment



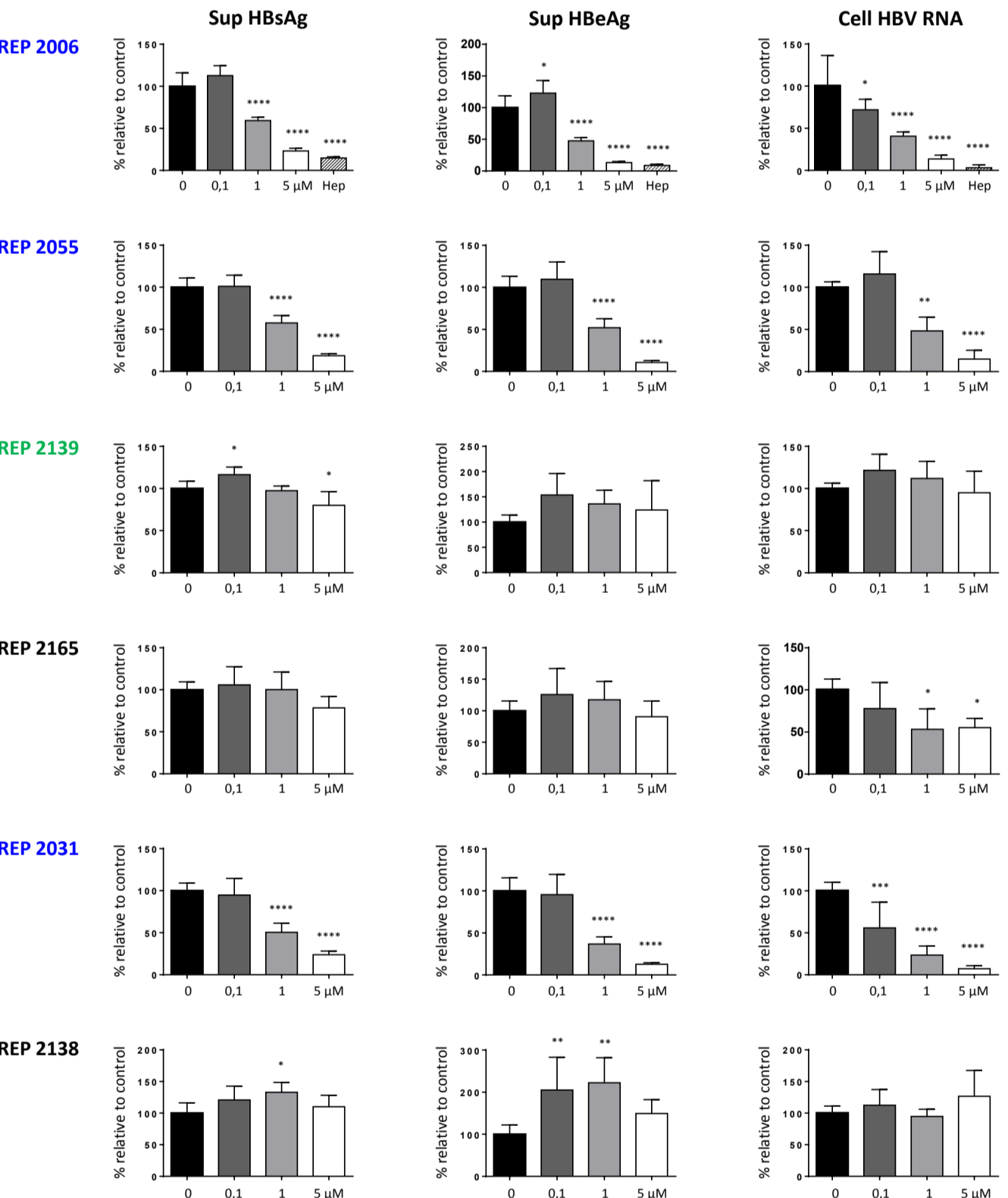
Post-treatment



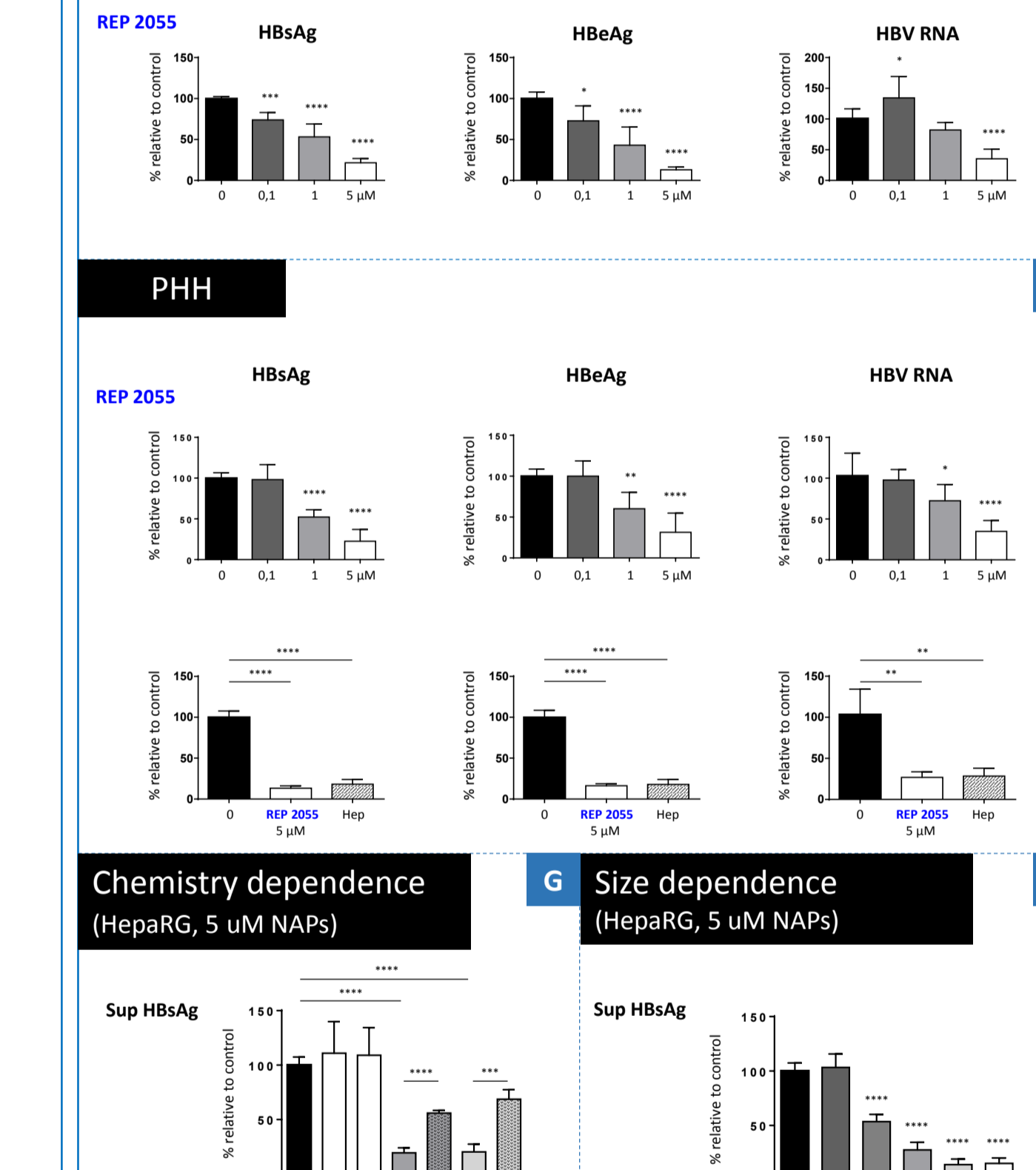
Co-treatment only



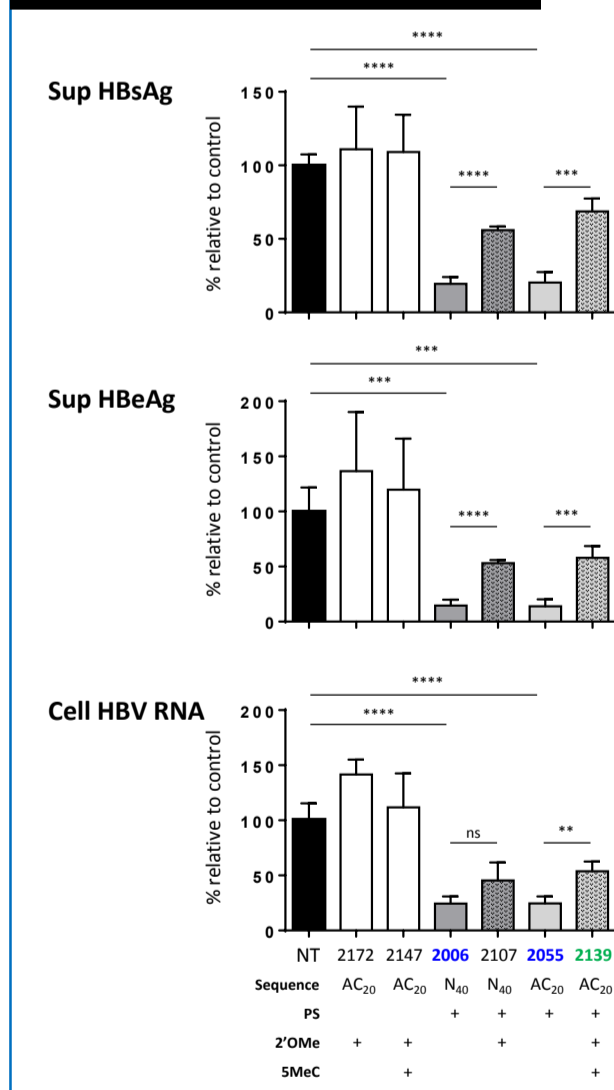
HepaRG



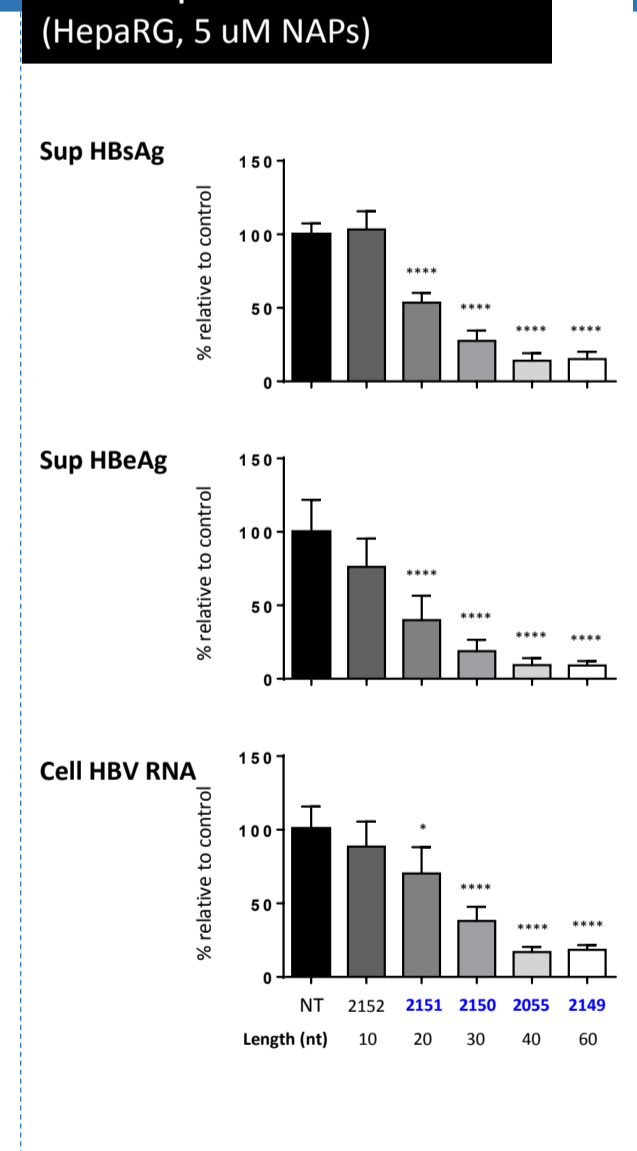
PHH



Chemistry dependence (HepaRG, 5 μM NAPs)



Size dependence (HepaRG, 5 μM NAPs)



- NAPs did not elicit any significant cytotoxicity, as monitored using a neutral red assay (data not shown)
- When added co + post viral inoculation, DNA based, phosphorothioated NAPs (e.g. REP 2006, REP 2055, and 2031) reduced the secreted HBsAg in both HepaRG (A) and PHH (B).
- HBsAg reductions were not observed with RNA based (2'O Me modified) phosphorothioated NAPs (e.g. REP 2139) or non phosphorothioated NAPs (e.g. REP 2138).**
- When added to the cells after viral inoculation NAPs did not alter the secretion of HBsAg (C,D). NAPs delivered with lipofection also had no effect (data not shown).
- REP 2055 present only during inoculation was sufficient to reduce the concentration of HBV cellular RNA as well as secreted HBsAg and HBeAg in a dose dependent manner (E,F). This observation was confirmed with other DNA based NAPs (G).
- The observed effects are sequence independent and do not rely on the activation of the innate immune response by CpG containing sequences (G).
- Phosphorothioation is mandatory for proper antiviral effect as shown by the inability of REP 2172, REP 2147 and REP 2138 to impair HBV lifecycle (G).
- The observed antiviral effects with DNA based NAPs at the time of viral inoculation is size dependent, with an optimal for a length of 40 nucleotides (H).

CONCLUSIONS & PERSPECTIVE

- NAPs inhibit HBV entry into HepaRG and PHH with an effect similar to other enveloped viruses:**
 - Entry effect is sequence independent.
 - Only amphipathic (phosphorothioated) NAPs are active.
 - NAPs >30 nucleotides are required, 40mer for optimal effect.
 - Polyanionic NAPs (non-phosphorothioated) are inactive.
- RNA NAPs (2'O Me modified) are uniquely inactive in blocking HBV entry including the clinically active REP 2139.**
- NAPs can block HBV entry, however, the antiviral effect of NAPs *in vivo* and in patients appears to be derived from a post-entry mechanism which is difficult to observe in HepaRG and PHH with treatment or lipofection.

REFERENCES

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