Benefit of interferon-free, nucleic acid polymer-based combination therapy for chronic hepatitis B

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BACKGROUND & AIMS

- Nucleic acid polymers (NAPs) are a promising new therapy for chronic hepatitis B treatment since they inhibit HBsAg release from infected hepatocytes.
- The clinical NAP compound, REP 2139, was shown to achieve the elimination of circulating HBsAg in human subjects with chronic HBV infection.

Ø The goal of this preclinical study was to examine the in vivo antiviral effect of combining REP 2139 with tenofovir disoproxil fumarate (TDF) and entecavir (ETV) in the chronic DHBV infection model.

MATERIAL & METHODS

- Three-day-old Pekin ducklings were infected with $2x10^{11}$ VGE/ml of DHBV from infectious duck serum
- Antiviral treatment was started in 28 days-old animals and lasted for 28 days using the following dosing regimens:
  - **REP 2139-Ca:** daily via 10mg/kg IP injection (REP 2139 formulated as a calcium chelate complex).
  - **ETV:** 1 mg daily/ oral gavage
  - **TDF:** 15mg daily / oral gavage
- Treatment groups (n=10) consisted of NS (IP) control, REP 2139-Ca, TDF, REP 2139-Ca + TDF and REP 2139-Ca + TDF + ETV.
- Antiviral activity was assessed by monitoring serum DHBsAg and anti-DHBpreS (anti-DHBsAg) antibodies by ELISA and serum and liver DHBV DNA and cccDNA by quantitative PCR.
- Viremia was assessed by qPCR at day 1, 14 and 28 of treatment and 4 and 8 weeks after the end of treatment (follow up). Liver DHBV DNA and cccDNA were assessed by qPCR at autopsy.
- Immunostaining of surface antigen (DHBsAg) was performed using primary 1H1 Mab and secondary HRP-conjugated sheep anti-mouse IgG.

CONCLUSIONS & PERSPECTIVE

ü **On-treatment antiviral performance of REP 2139 can be improved in the presence of TDF or ETV.**

ü **Importantly after 2 months off combination therapy (REP 2139 with TDF or TDF and ETV) a marked decrease in viral replication was still observed in large majority of animals indicating sustained virologic response** (SVR).

ü **Interestingly ducks with SVR exhibiting low total and cccDNA at the end of follow-up had also undetectable liver DHBsAg as assessed by immunostaining.**

Ø **An interferon-free regimen of REP 2139 with TDF or ETV could lead to improved antiviral outcomes or allow the shortening of antiviral regimens in patients with chronic HBV infection.**

REFERENCES

1. Noordeen et al., 2013 Antimicrob Agents Chemother. 57: 5291-5298.
2. Noordeen et al., 2013 Antimicrob Agents Chemother. 57: 5299-5406.
3. Noordeen et al., 2015 PLoS ONE 10: e0140909.

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RESULTS

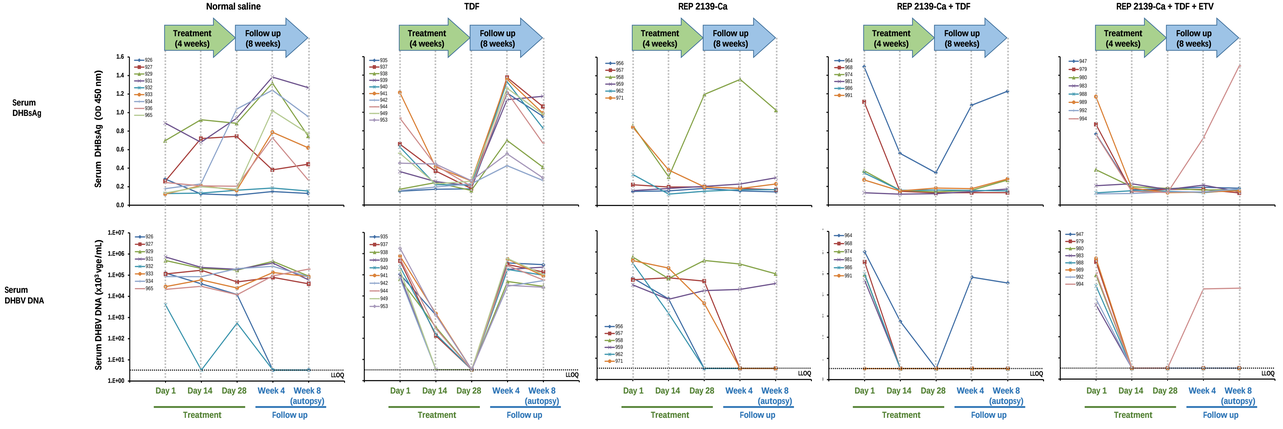


Figure 1. Effect of NAP-based combination therapy on viremia and DHBsAg in DHBV-infected Pekin ducks. Serum DHBV DNA and DHBsAg analysis during 28 days of treatment and 8 weeks follow-up after end of treatment. Sampling points are indicated on X-axis (not to scale). A rebound of viral replication was observed in all ducks after TDF monotherapy cessation. In contrast, combined therapy with REP 2139-Ca+TDF or REP 2139-Ca+TDF+ETV produced a more rapid antiviral effect on treatment and elicited a sustained virologic responses off-treatment in a majority of animals.

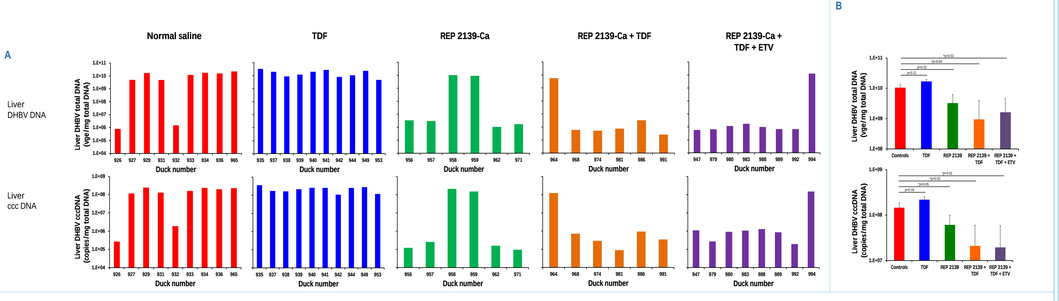


Figure 2. Effect of NAP-based combination therapy on DHBV in the liver of infected Pekin Ducks. Analysis of liver DHBV DNA and cccDNA at end of follow up. Reductions in liver DHBV DNA and cccDNA in individual animals (A) and among treatment groups (B) were observed in REP 2139-Ca treated ducks, but not in TDF-monotherapy group, and were only found in animals who experienced a sustained virologic response after treatment (SVR). In (B) statistically significant differences between group means (total and cccDNA) are indicated (*) as determined by Mann-Whitney U test.

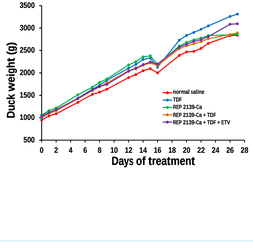
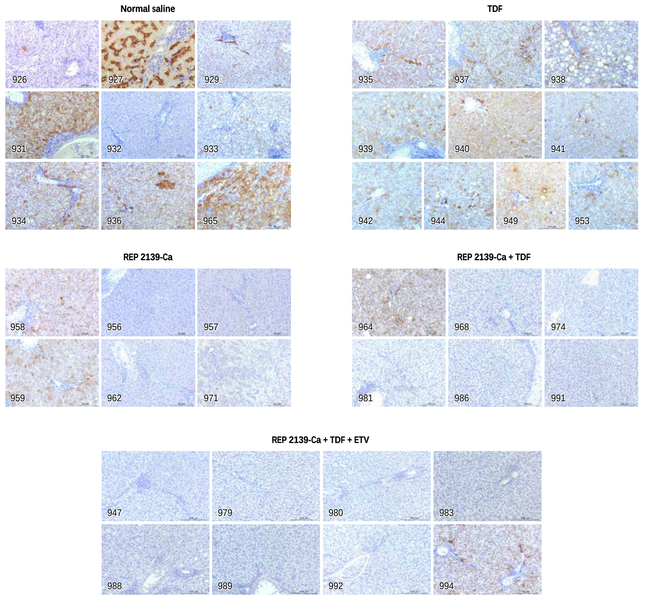


Figure 3. Mean group body weights during treatment.
No weight loss was observed in any treatment group, indicating absence of adverse effect



Summary : semi-quantitative score. % of DHBsAg-positive / total hepatocytes

	-	+	+/++	++	++/+++	+++	Total
	0%	1-37%	38-50%	51-60%	61-80%	85-100%	
N saline	1	2	4	0	1	1	9
TDF	-	2	5	3	-	-	10
REP2139	4	-	-	-	2	-	6
REP2139 + TDF	5	-	1	-	-	-	6
REP2139 + TDF+ ETV	7	-	1	-	-	-	8

Figure 4. Detection of DHBsAg positive hepatocytes by immunostaining of autopsy liver samples. All animals from TDF-monotherapy group exhibited detectable DHBsAg by immunostaing. By contrast SVR from REP 2139-Ca treated groups had no liver DHBsAg (40x magnification).