Achievement of surface antigen clearance in the liver by combination therapy with REP 2139-Ca and nucleoside analogues against chronic hepatitis B

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BACKGROUND

- 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-Ca, was shown to achieve the elimination of circulating HBsAg in human subjects with chronic HBV infection.

OBJECTIVES

- The aim of this preclinical study was to assess the antiviral effect of REP 2139-Ca in combination with tenofovir disoproxil fumarate (TDF) and entecavir (ETV) on makers of chronic DHBV infection *in vivo*.
- **We focused on the ability of this novel combination therapy to clear viral surface antigen in the liver**.

MATERIALS & METHODS

- Chronic DHBV-carrier ducks, infected as neonates, were randomized into 4 groups (n=10):
- NS (controls); monotherapy REP 2139-Ca or TDF; combination therapy REP 2139-Ca + TDF or REP 2139-Ca + TDF & ETV
- Antiviral treatment started in 28 days-old animals and lasted for 4 weeks 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
- Importantly, all animals were followed during additional 8 weeks after treatment cessation
- Antiviral activity was assessed by monitoring serum DHBsAg and anti-DHBpreS (anti-DHBsAg) antibodies by ELISA and serum DHBV DNA by qPCR, liver DHBV DNA and cccDNA by qPCR
- ☐ Sustained functional control of infection (**FC**) was defined as stable suppression of serum DHBsAg and DHBV DNA during 2 months off-therapy
- Immunostaining of surface antigen (DHBsAg) was performed using primary 1H1 Mab and secondary HRP-conjugated sheep anti-mouse IgG.

RESULTS

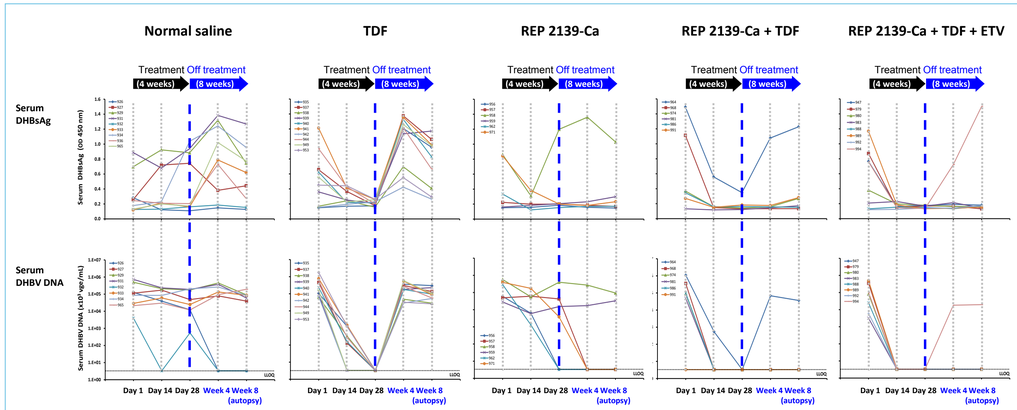

Figure 1. Effect of NAP-based combination therapy on viremia and DHBsAg in DHBV-infected Pekin ducks. Serum DHBV DNA and DHBsAg analysis during 28 days of treatment and 8 weeks follow-up after end of treatment. Sampling points are indicated on X-axis (not to scale). A rebound of viral replication was 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 functional control (FC) off-treatment in a majority of animals.

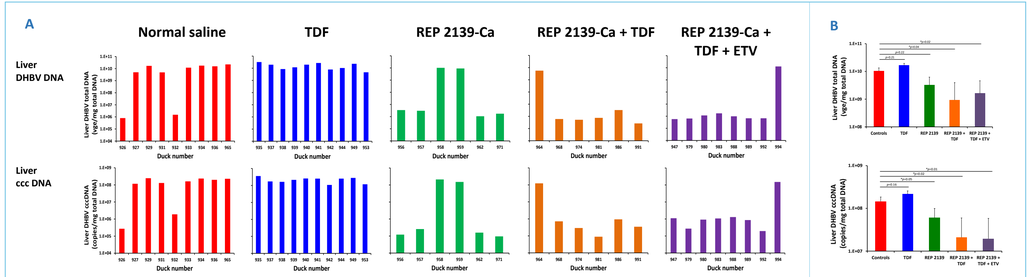

Figure 2. Effect of NAP-based combination therapy on DHBV in the liver of infected Pekin Ducks. Analysis of liver DHBV DNA and cccDNA at end of 8-weeks follow up. Sustained reductions in liver DHBV DNA and cccDNA in individual animals (A) and among treatment groups (B) were observed in REP 2139-Ca treated ducks, but not in TDF-monotherapy group, and were only found in animals who experienced a sustained functional control of infection (FC). In (B) statistically significant differences between group means (total and cccDNA) are indicated (*) as determined by Mann-Whitney U test.

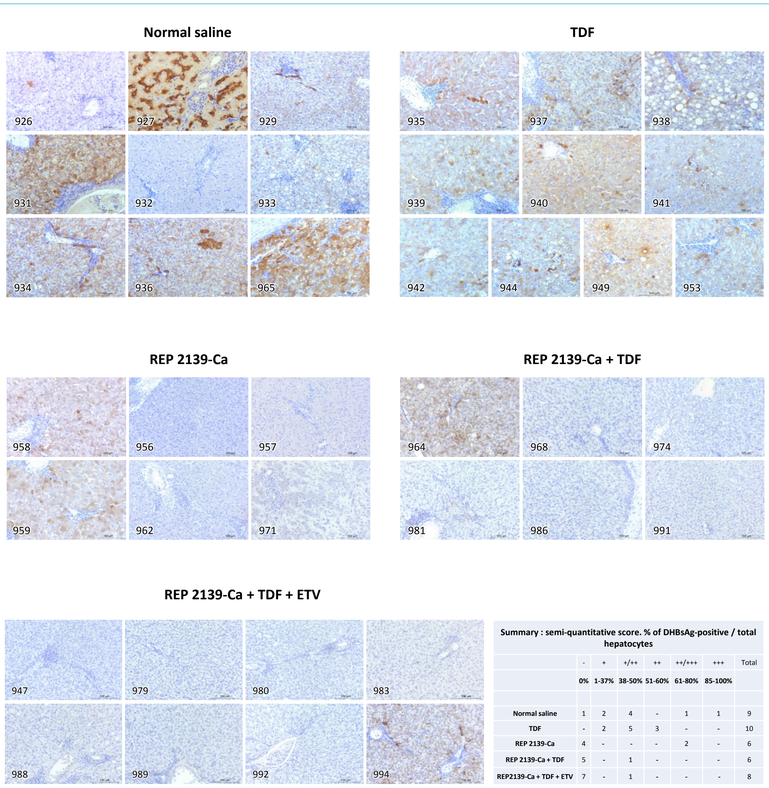

Figure 4. Detection of DHBsAg positive hepatocytes by immunostaining of autopsy liver samples. All animals from TDF-monotherapy group exhibited detectable DHBsAg by immunostaining. By contrast FC from REP 2139-Ca treated groups had no liver DHBsAg (40x magnification).

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

	-	+	+/++	++	++/+++	+++	Total
	0%	1-37%	38-50%	51-60%	61-80%	85-100%	
Normal saline	1	2	4	-	1	1	9
TDF	-	2	5	3	-	-	10
REP 2139-Ca	4	-	-	-	2	-	6
REP 2139-Ca + TDF	5	-	1	-	-	-	6
REP2139-Ca + TDF + ETV	7	-	1	-	-	-	8

CONCLUSIONS

- ✓ Combination therapy led to sustained functional control (FC) of infection as demonstrated by a significant decrease of total viral liver DNA and cccDNA in a large majority of animals 2 months after treatment cessation as compared with TDF-monotherapy.
- ➢ **Importantly, combination therapy resulted also in the clearance of surface Ag in the liver of all animals exhibiting FC**.
- ➢ **IFN-free regimen combining REP 2139-Ca with TDF or TDF & ETV led to dramatic reduction or clearance of all markers of viral infection, including liver DHBsAg.**
- ✓ Synergistic antiviral effects were observed when REP 2139-Ca was combined with TDF or TDF & ETV.

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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