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An in vivo duck hepatitis B virus model recapitulates key aspects of nucleic acid polymer treatment outcomes in chronic hepatitis B patients

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ALIGOS THERAPEUTICS

Background and aims

Nucleic acid polymers (NAPs) are an attractive treatment modality for chronic hepatitis B (CHB), with REP2139 and REP2165 having shown efficacy in CHB patients.[1] A significant proportion of patients achieve functional cure, whereas the others exhibit a moderate response or are non-responders. NAP efficacy has been difficult to recapitulate in animal models, with the duck hepatitis B virus (DHBV) model showing some promise but remaining underexplored for NAP efficacy testing.[2] Here we describe an optimized in vivo DHBV duck model and explore several characteristics of NAP treatment in this model.

Methods

Pekin ducks (*Anas platyrhynchos domesticus*) were intravenously injected with DHBV-containing serum shortly after hatching. A blood sample was obtained before inoculation and analyzed for DHBV DNA to exclude endogenously infected ducklings. After establishment of infection, animals were treated with entecavir, REP2139 and/or REP2165 and serum DHBV DNA and DHBV surface antigen (DHBsAg) levels were determined weekly. Animals were followed up for several weeks after end of treatment. NAP serum and tissue concentrations were determined by mass spectrometry.

Subcutaneous dosing of REP2139 is efficacious in DHBV-infected ducks

DHBV-infected ducks were randomized and treated with entecavir (1 mg/day, PO) or REP2139, comparing intraperitoneal (IP) dosing with subcutaneous (SC) dosing. Ducks were dosed with 10 mg/kg of REP2139: QD for 14 days, and then switched to dosing every other day (QOD) for the remaining 14 days for reasons of tolerability. After the end of treatment, animals were followed up for an additional 8 weeks. Serum samples were obtained weekly and DHBV DNA and DHBsAg levels were quantified. As shown in Figure 1, untreated animals showed relatively stable viral titers over time, with DHBsAg being more variable than DHBV DNA. In general, DHBV DNA titers correlated well with DHBsAg levels (data not shown). Entecavir induced a uniform decline in DHBV DNA levels, followed by a gradual rebound after end of treatment. REP2139 induced a sustained response on both DHBV DNA and DHBsAg in approximately half the treated animals, with a clear distinction between responders and non-responders, in line with earlier results from duck studies and clinical trials in CHB patients.[1,2] Animals with lower baseline titers were more likely to respond to treatment. We show for the first time that a NAP is also efficacious in the DHBV duck model when dosed subcutaneously.

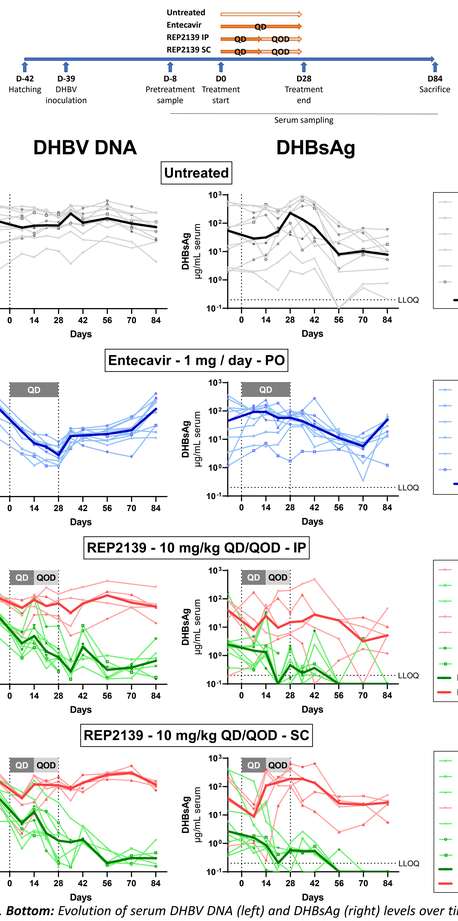

Figure 1 – Top: *Study schematic.* ***Bottom:*** *Evolution of serum DHBV DNA (left) and DHBsAg (right) levels over time in untreated ducks, and ducks treated with entecavir or REP2139 (either IP or SC). Curves and values represent individual ducks. IP, intraperitoneal; PO, per os; QD, every day; QOD, every other day; SC, subcutaneous.*

REP2139 serum concentrations do not correlate with response

Quantification of REP2139 serum and tissue levels showed slightly higher concentrations in serum and kidney for SC dosing, compared to IP dosing, but similar liver levels. Serum REP2139 concentrations decreased somewhat when the dosing frequency was reduced from QD to QOD, as expected. Interestingly, REP2139 serum concentrations did not correlate with virological response, as assessed by comparing DHBV declines from baseline at day 14 and day 21 (Figure 2). The appearance of anti-DHBsAg antibodies was observed occasionally in both untreated and REP2139-treated animals but did not correlate with efficacy (data not shown).

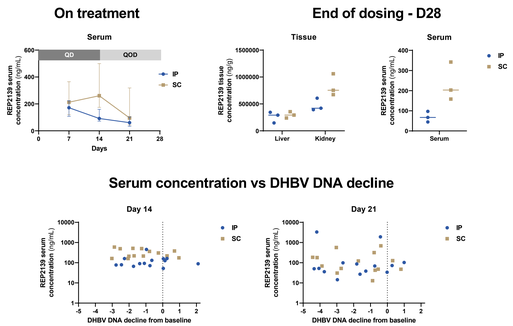

Figure 2 – Top left: *On-treatment REP2139 serum concentrations over time. Values represent median ± 95% CI from 14 ducks per group.* ***Top right:*** *REP2139 concentrations in liver, kidney, and serum at the end of treatment. Values represent individual ducks.* ***Bottom:*** *REP2139 serum concentrations vs DHBV DNA decline from baseline after 14 (left) and 21 (right) days of dosing. Values represent individual ducks.*

Endogenously DHBV-infected ducklings do not respond to REP2139 treatment

In a subsequent experiment, ducklings were obtained that were endogenously infected with DHBV (through vertical transmission), as confirmed by serum sampling just after hatching and DHBV DNA determination. Ducks were either untreated or treated with entecavir (1 mg/day, PO) or REP2139 (10 mg/kg QOD, SC) for 28 days and followed up for an additional 27 days. Interestingly, none of the ducks responded to REP2139, whereas entecavir resulted in the expected pronounced DHBV DNA decline on treatment, followed by rebound post treatment withdrawal (Figure 3). The lack of efficacy for REP2139 in this setting suggests a role for the immune system in NAP efficacy, at least in the DHBV duck model.

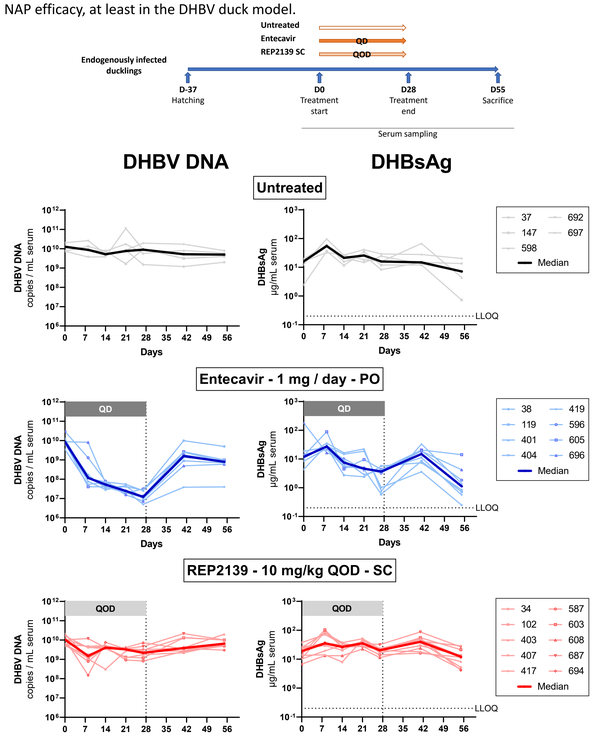

Figure 3 – Top: *Study schematic.* ***Bottom:*** *Evolution of serum DHBV DNA (left) and DHBsAg (right) levels over time in untreated ducks, and ducks treated with entecavir or REP2139 (SC). Curves and values represent individual ducks. Endogenous infection was confirmed in all ducks.*

Entecavir pretreatment increases REP2139 response rates

Since animals with lower baseline viral titers tended to be more likely to respond to REP2139, we investigated whether an initial lowering of viral titers through entecavir pretreatment for two weeks, followed by REP2139 add-on, would improve virological response. Indeed, 4/5 ducks in the add-on group showed a sustained response for DHBV DNA and DHBsAg (Figure 4), as opposed to only 50% in the initial monotherapy study.

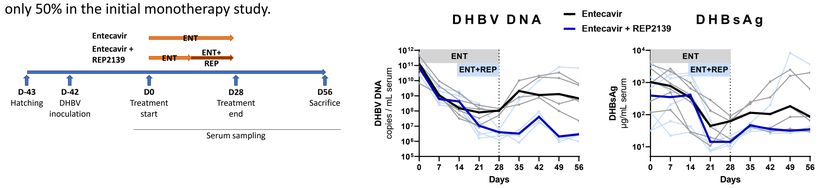

Figure 4 – Left: *Study schematic.* ***Right:*** *Evolution of serum DHBV DNA (left) and DHBsAg (right) levels over time ducks treated with entecavir only or with entecavir for 14 days, followed by entecavir and REP1239 for the next 14 days. Light curves and values represent individual ducks, bold curves represent the median of each treatment group.*

Destabilized REP2165 has different virological response kinetics than REP2139

NAP REP2165 is an analogue of REP2139 with accelerated clearance, due to the introduction of 3 riboadenosines lacking 2'-O-methylation.[1] In a head-to-head comparison of REP2139 and REP2165 in the DHBV duck model, REP2165 demonstrated a distinctly different virological profile, as shown in Figure 5. REP2139 showed the characteristic early divergence of responders and non-responders (albeit with a lower response rate compared to our first study), while REP2165 exhibited a much more homogeneous response on treatment, followed by almost uniform rebound, with a single responding duck as the exception. Quantification of liver NAP concentrations at end of treatment and end of follow-up confirmed the accelerated clearance of REP2165 compared to REP2139 (data not shown).

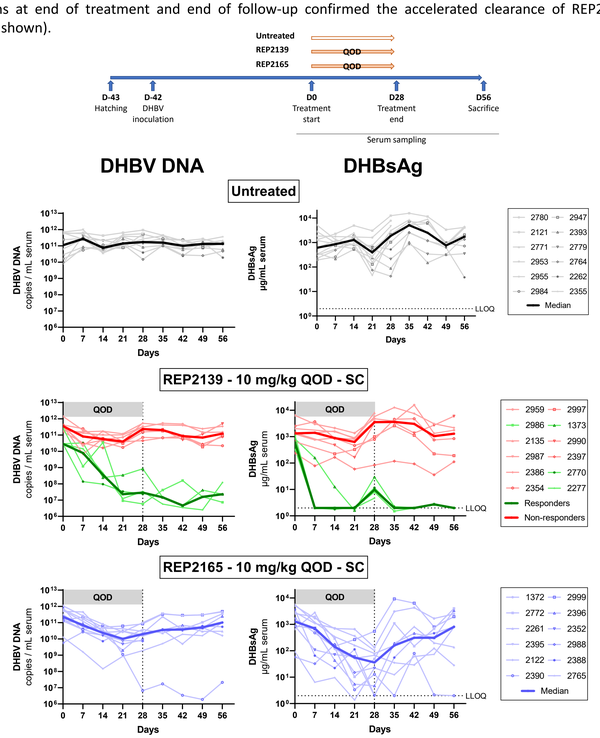

Figure 5 – Top: *Study schematic.* ***Bottom:*** *Evolution of serum DHBV DNA (left) and DHBsAg (right) levels over time in untreated ducks, and ducks treatment with REP2139 or REP2165. Curves and values represent individual ducks.*

Conclusions

Subcutaneous administration of NAPs leads to a pronounced antiviral effect in the DHBV duck model, with a clear distinction between responders and non-responders. Interestingly, endogenously infected ducklings do not respond to REP2139 and NAP liver concentrations do not correlate with response. The DHBV duck model provides a useful tool for in vivo evaluation of NAPs, recapitulating many aspects of this class of compound's efficacy in CHB patients. We have utilized the model for further explorations of this class of compounds and will report on those studies in the future.

References: [1] Bazinet et al 2020 Gastroenterology, 158: 2180-94. [2] Quinet et al 2018 Hepatology, 67: 2127-40.

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