Background and aims
Nucleic acid polymer (NAP) is 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 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/kg/day, PO) or REP2139, comprising 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 (QD2) 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 and in DHBV-infected ducklings, almost 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. 3 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.

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 QD2, 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).

Endogenously DHBV-infected ducks 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 (5 mg/kg/day, PO) or REP2139 (10 mg/kg/day, QD) 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 task of efficacy for REP2139 in this setting suggests a role for the immune system in NAP efficacy, at least in the DHBV duck model.

Entecavir pretreatment increases REP2139 response rates
Since animals with lower baseline viral titer tended to be more likely to respond to REP2139, we investigated whether an initial braving of virion titers through entecavir pretreatment for two weeks, followed by REP2139 add-on, would improve virological response. Indeed, 6/7 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.

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 ducks do not respond to REP2139 and NAP response kinetics recapitulating many aspects of this class of compound’s efficacy in CHB patients. We have utilized the model for further assessment of the pharmacodynamics and safety of REP2139 and REP2165.

References
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Figure 1 – Top: Study schematic. Animals with lower baseline titers were more likely to respond to treatment. Interestingly, endogenously infected ducks did not respond to REP2139 treatment. (A) Animals with lower baseline titers were more likely to respond to treatment. (B) Endogenously infected ducks do not respond to REP2139 treatment. (C) Untreated untreated ducks 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).

Figure 2 – Top: Study schematic. (A) REP2139 serum concentrations do not correlate with response. Serum concentration vs DHBV DNA decline. Values represent individual ducks. (B) REP2139 serum concentrations do not correlate with response. Serum concentration vs DHBV DNA decline. Values represent individual ducks.

Figure 3 – Top: Study schematic. (A) Endogenously DHBV-infected ducks do not respond to REP2139 treatment. Serum concentration vs DHBV DNA decline. Values represent individual ducks. (B) Endogenously DHBV-infected ducks do not respond to REP2139 treatment. Serum concentration vs DHBV DNA decline. Values represent individual ducks.

Figure 4 – Left: Study schematic. Right: Evolution of serum DHBV DNA (left) and DHBsAg (right) levels vs time in untreated ducks, and ducks treated with entecavir or REP2139. (C) Untreated untreated ducks 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).