



A human monoclonal antibody against HBsAg for the prevention and treatment of chronic HBV and HDV infection

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Background & Aims: Elimination of chronic HBV/HDV infection remains a major global health challenge. Targeting excessive hepatitis B surface antigen (HBsAg) release may provide an interesting window of opportunity to break immune tolerance and to achieve a functional cure using additional antivirals.

Methods: We evaluated a HBsAg-specific human monoclonal antibody, as part of either a prophylactic or therapeutic strategy, against HBV/HDV infection in cell culture models and in human-liver chimeric mice. To assess prophylactic efficacy, mice were passively immunized prior to infection with HBV or HBV/HDV (coinfection and superinfection setting). Therapeutic efficacy was assessed in HBV and HBV/HDV-coinfected mice receiving 4 weeks of treatment. Viral parameters (HBV DNA, HDV RNA and HBsAg) were assessed in mouse plasma.

Results: The antibody could effectively prevent HBV/HDV infection in a dose-dependent manner with IC₅₀ values of ~3.5 ng/ml. Passive immunization showed complete protection of mice from both HBV and HBV/HDV coinfection. Moreover, HDV superinfection was either completely prevented or at least attenuated in HBV-infected mice. Finally, antibody treatment in mice with established HBV/HDV infection resulted in a significant decline in viremia and a concomitant drop in on-treatment HBsAg, with a moderate viral rebound following treatment cessation.

Conclusion: We present data on a valuable antibody candidate that could complement other antivirals in strategies aimed at achieving functional cure of chronic HBV and HDV infection.

Impact and implications: Patients chronically infected with HBV may eventually develop liver cancer and are at great risk of being superinfected with HDV, which worsens and accelerates disease progression. Unfortunately, current treatments can rarely eliminate both viruses from chronically infected patients. In this study, we present data on a novel antibody that is able to prevent chronic HBV/HDV infection in a mouse model with a humanized liver. Moreover, antibody treatment of HBV/HDV-infected mice strongly diminishes viral loads during therapy. This antibody is a valuable candidate for further clinical development.

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Introduction

Despite the availability of safe and efficacious HBV vaccines, approximately 300 million people worldwide are currently chronic carriers of hepatitis B surface antigen (HBsAg).¹ Chronic HBV infection may eventually lead to cirrhosis and hepatocellular

carcinoma (HCC) development. HBV is responsible for a heavy disease burden and an estimated 820,000 liver-related deaths annually.^{2,3} Moreover, at least 12 million and up to 60 million patients with chronic HBV are estimated to suffer from a concomitant HDV infection.^{4–7} Since HDV infection greatly exacerbates disease progression by rapidly inducing cirrhosis, liver dysfunction and HCC, it is considered the most severe form of viral hepatitis.⁵ Patients coinfecting with HBV and HDV usually recover spontaneously through immune-mediated viral elimination. However, in 80% of cases, patients with chronic HBV who become superinfected with HDV progress to a chronic disease state, resulting in rapid deterioration of the pre-existing HBV-related liver damage and very high mortality rates.⁸

Current lifelong HBV therapy with nucleos(t)ide analogues (NAs) suppresses viral replication, but only about 10% of all

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treated HBeAg-positive patients (and 1% in HBeAg-negative patients) may achieve a functional cure, *i.e.* complete HBsAg loss with/without seroconversion after a 5-year follow-up.^{9–11} Therefore, NAs will not eliminate the risk of cancer development. Regarding HDV therapy, this virus does not code for polymerases or proteases that could be therapeutically targeted as is the case for HBV or HCV, but relies on both the host replicative machinery and on the helper function of HBV to complete its life cycle.¹² Therefore, the only recommended clinical regimen remains pegylated-IFN α , a therapy showing poor efficacy and infrequent long-term responses.¹³ Specifically, only 28% of treated patients achieved clearance of serum HDV 6 months after cessation of a 48-week regimen and 50% of responders experienced late HDV RNA relapse during longer follow-up.^{14–16} While phase III results are still pending, in 2020 the European Medicines Agency conditionally approved the entry-inhibitor Myrcludex B (bulevirtide), a preS1-derived peptide able to block the HBV/HDV host receptor sodium taurocholate co-transporting polypeptide (NTCP), for the treatment of patients with compensated chronic HDV infection.^{17–20} Several other HDV treatment options are currently being assessed in clinical trials.²¹ However, it is widely accepted that a combination of antiviral compounds targeting different steps in the viral life cycle will be required to eliminate both viruses.

In this study, we evaluated the capacity of a previously generated HBsAg-specific human monoclonal antibody (hu-mAb) to prevent and treat HBV and HDV infection both *in vitro* and *in vivo*.²² Targeting the entry-step in combination with diminishing circulating HBsAg would simultaneously block viral spread and potentially provide a window of opportunity for other antivirals to break immune tolerance in the context of chronic infection.

Materials and methods

More details can be found in the supplementary materials and methods.

Human monoclonal anti-HBsAg production and purification

The human monoclonal anti-HBsAg-specific antibody was produced using classical hybridoma technology, more specifically by fusion of SCID-engrafted human peripheral blood lymphocytes isolated from a vaccinated individual (6,981 mIU/ml anti-HBsAg at time of blood donation) with K6H6/B5 heteromyeloma cells as previously described.²² Fused cells were then seeded in culture medium supplemented with hybridoma growth factors and the selection drugs hypoxanthine-aminopterin-thymidine and ouabain. Anti-HBs-positive cultures were sequentially cloned and several monoclonal hybridoma lines were isolated and confirmed via commercial anti-HBs ELISA (DiaSorin, Italy). The hu-mAb was then purified from collected supernatant using conventional Protein G columns (Hitrap Protein G HP, Sigma Aldrich, USA). After evaluation by serum protein electrophoresis (SAS-MX Serum Protein-10, Sysmex, Japan), the antibody was concentrated (Amicon Ultra-15 50k, Sigma Aldrich, USA) and its concentration was determined using spectrophotometry at 280 nm.

In vitro prevention of HBV and HDV infection

For *in vitro* prevention of HBV infection, anti-HBsAg was applied to HepG2.hNTCP cells in duplicate at 5-fold serial dilutions ranging from 10 μ g/ml to 0.128 ng/ml. Two hours later, cells were infected with HBV (4,990 IU/cell; genotype D) and after 1 week, infection was assessed using immunofluorescent (IF) staining of

hepatitis B core antigen-positive cells. Images were captured by automated spinning disk microscopy using a 40x objective (CSU-X1, Nikon). Per condition, a 20 x 10 field was captured (in duplicate) and positive cells were automatically counted using ImageJ software v1.53c. *In vitro* prevention of HDV infection (genotype 1) was examined in the same manner, but in three different cell lines (HepG2.hNTCP, Huh7.5.hNTCP and NEB2.7). Imaging was performed using the Leica TCS-SPE microscope with a 20x objective. Per condition, three random pictures were taken (in duplicate) and HDV-positive cells were automatically counted using ImageJ software v1.53c. More details are provided in the supplementary materials and methods.

Mice

All mice were bred under sterile conditions and all experiments were approved by the Animal Ethics Committee of the Faculty of Medicine and Health Sciences of Ghent University. Human-liver chimeric mice were generated by transplanting 10⁶ primary human hepatocytes (donor C342, C399 and HH223 from Corning, the Netherlands; and donor L191501 from Lonza, Switzerland) into homozygous uPA^{+/+}-SCID mice as previously described.^{23,24} Human albumin was quantified in mouse plasma to evaluate successful humanization of the mouse liver using conventional ELISA (Bethyl Laboratories, USA). Mice with albumin levels ranging between 2–10 mg/ml were selected for this study and groups were randomized, taking into consideration human albumin levels, infection levels and general condition (*e.g.* based on body weight). Mice were 8- to 9-weeks-old at the start of infection.

In vivo prevention of HBV, HBV/HDV and HDV superinfection

For *in vivo* prevention of HBV, human-liver chimeric mice (*n* = 6) repopulated with human hepatocytes from two different donors (*n* = 3 for C342 and *n* = 3 for HH223) were passively immunized through intraperitoneal (IP) administration of 1 mg hu-mAb 3 days before challenge with HBV (patient serum, 10⁶ IU/mouse). As controls, six additional mice were infected without prior passive immunization. For prevention of HBV/HDV coinfection, humanized mice (*n* = 4) engrafted with hepatocytes from donor C342 were passively immunized (1 mg hu-mAb, IP) 3 days prior to HBV/HDV coinfection with cell culture-derived virus (5 x 10⁶ IU HBV DNA/mouse and 2.3 x 10⁶ IU HDV RNA/mouse). As controls, three additional mice were coinfecting with HBV/HDV without prior passive immunization. Mice (co-)infected with HBV or HBV/HDV were monitored until week 12–13 post-inoculation. Finally, for prevention of HDV superinfection, humanized mice were first infected with either patient-derived HBV (10⁶ IU/mouse, *n* = 7, mice engrafted with donor L191501 hepatocytes) or cell culture-derived HBV (5 x 10⁶ IU per mouse, *n* = 14, *i.e.* five donor C342 and nine C399 engrafted mice). Mice were superinfected with patient or cell culture-derived HDV (both 2.55 x 10⁵ IU/mouse) 6 or 8 weeks later, respectively. Three days prior to superinfection, 5/7 mice infected with patient-derived HBV and 9/14 mice infected with cell culture-derived HBV were passively immunized with 1 mg of hu-mAb IP. Mice superinfected with HDV were monitored for 9–10 weeks following HDV inoculation. Blood plasma was collected every 1–2 weeks (depending on the condition of the mouse and the timing post-infection) and HBV DNA and HDV RNA levels were quantified via commercial HBV/HDV RealStar[®] (reverse transcription-) qPCR (Altona Diagnostics, Germany) or the RealTime m2000 HBV assay (Abbot, USA). Anti-HBsAg ELISA was performed using a

commercially available quantitative kit (Beijing Wantai Biological, China).

Treatment of HBV and HBV/HDV (co-)infected mice

Human-liver chimeric mice ($n = 7$) transplanted with donor C342 hepatocytes were chronically infected with cell culture-derived HBV (5×10^6 IU/mouse). Four out of seven mice received 1 mg hu-mAb (IP) twice a week for 4 consecutive weeks (eight doses in total), starting at week 11 post viral inoculation. The remaining three infected mice were left untreated as controls. In a second treatment experiment, 13 human-liver chimeric mice transplanted with donor L191501 hepatocytes were first infected with cell culture-derived HBV (5×10^6 IU/mouse) before being superinfected with cell culture-derived HDV (2.55×10^5 IU/mouse) 6 weeks later. At week 10, 7/13 mice received 1 mg hu-mAb (IP) twice a week for 4 consecutive weeks (nine doses in total). The six remaining coinfecting mice did not receive any treatment (control). Blood plasma was collected every 1 to 2 weeks (depending on the condition of the mouse and the timing post-infection) and the levels of HBV DNA, HDV RNA and hu-mAb were determined as described above. Plasma samples were analyzed by SDS-PAGE and western blot was used to assess HBsAg levels.

SDS-PAGE and western blot analysis

Exactly 0.5 μ l of mouse plasma was used for sample preparation and denaturation (70 °C, 10 min), and subjected to SDS-polyacrylamide gel (12%) electrophoresis, with proteins transferred to a PVDF membrane. Membranes were blocked (5% skim milk in TBS-T) and HBsAg was detected by a primary goat anti-HBsAg antibody (70-HG15; Fitzgerald industries; 1/1,000), followed by a horse radish peroxidase-conjugated rabbit anti-goat antibody (31402; Thermo Fisher Scientific; 1/20,000). Immunoblots were developed using the SuperSignal™ West Femto Maximum Sensitivity Substrate kit (Thermo Fisher Scientific, USA) and exposed to the ImageQuant LAS4000 chemiluminescent imaging system (GE Healthcare, Diegem, Belgium). More details are provided in the supplementary materials and methods.

Statistical analysis

GraphPad Prism v9.3.1 was used for graph visualization and statistical analyses. IC_{50} values were calculated using non-linear regression (curve fit). Normality tests and Mann-Whitney U tests enabled comparisons between normalized log mean virus levels of treated vs. non-treated mice. Data were considered statistically significant if $p < 0.05$.

Results

In vitro prevention of HBV infection

The HBV-permissive cell line HepG2.hNTCP was (pre-)incubated with 5-fold serial antibody dilutions before, during and after viral inoculation. Infected cells were visualized via immunofluorescent staining (hepatitis B core antigen) after 1 week. Near complete abrogation of infection was shown when at least 80 ng/ml hu-mAb was applied, while an overall dose-dependent inhibitory effect was observed with an IC_{50} value of 3.531 ng/ml (Fig. 1).

In vitro prevention of HDV infection

The HDV inoculum was pre-incubated with 5-fold serial hu-mAb dilutions and three cell lines were subjected to this virus-antibody mixture. Antibodies were maintained for 1 week until visualization of infected cells by IF. As shown in Fig. 2, infection was nearly completely abrogated using at least 16 ng/ml hu-mAb for all three cell lines tested. A clear dose-dependent inhibitory effect was observed with IC_{50} values of 3.275 ng/ml, 4.393 ng/ml and 3.080 ng/ml for HepNB2.7, HepG2.hNTCP and Huh7.5.hNTCP cells, respectively.

In vivo prevention of HBV infection

Six human-liver chimeric mice (repopulated with hepatocytes from two distinct donors) were passively immunized (1 mg hu-mAb/mouse) 3 days prior to HBV inoculation. As controls, six animals were injected with the virus without prior passive immunization. In donor C342 engrafted mice, no virus was detected in the three passively immunized mice until at least week 6 (on which two were found dead) and one mouse experienced HBV DNA breakthrough at week 10, while the controls showed strongly

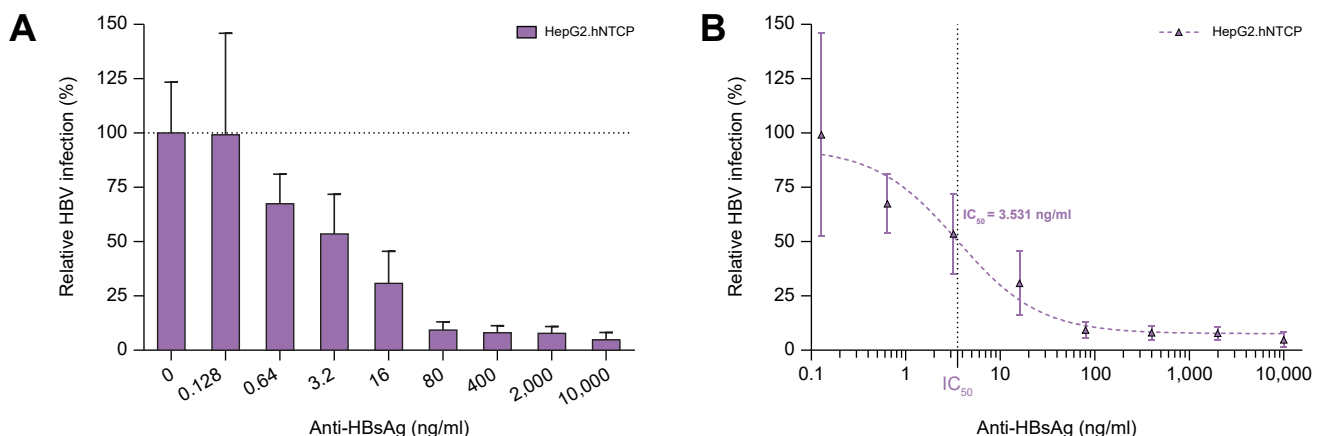


Fig. 1. In vitro prevention of HBV infection. (A) A 5-fold serial dilution of hu-mAb was evaluated for the prevention of HBV infection in HepG2.hNTCP cells. Infectivity was assessed using hepatitis B core antigen immunofluorescence staining 1 week after infection. Relative infection (%): total number of infected cells relative to the control condition where no antibody was applied. (B) The IC_{50} value was calculated from the antibody dose-response curve by non-linear regression (curve fit). All conditions were performed in duplicate. All data represented as mean \pm SD (error bars). hu-mAb, human monoclonal antibody; IC_{50} , half maximal inhibitory concentration.

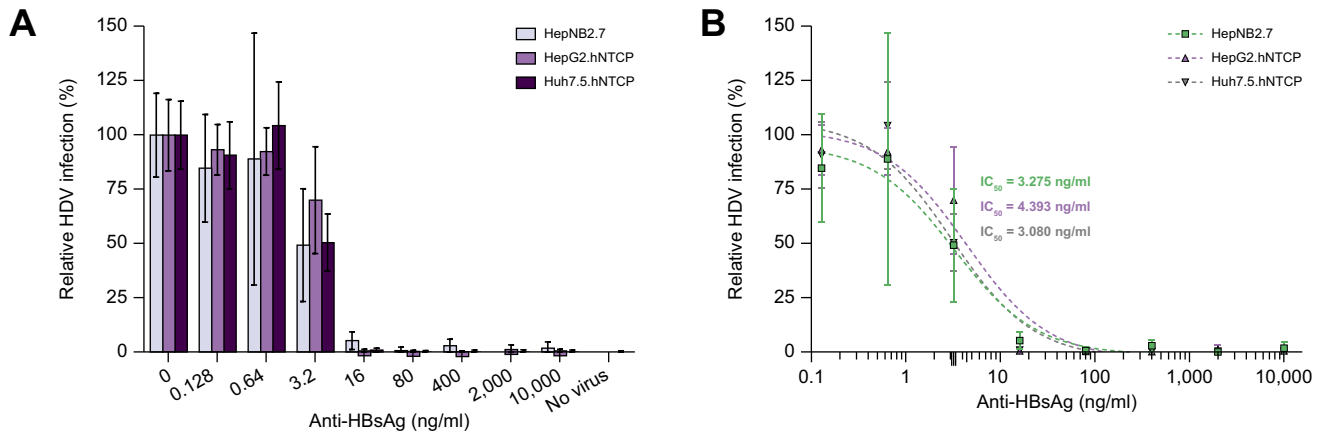


Fig. 2. In vitro prevention of HDV infection. (A) A 5-fold serial dilution of hu-mAb was evaluated for the prevention of HDV infection in HepNB2.7 (grey), HepG2.hNTCP (purple) and Huh7.5.hNTCP (dark purple) cells. The hu-mAb was pre-incubated with the HDV inoculum before challenge and the antibody was maintained for 7 days until HDAG immunofluorescence staining. Relative infection (%): total number of infected cells relative to the control condition where no antibody was applied. (B) The IC_{50} values were calculated from the antibody dose-response curves by non-linear regression (curve fits). Baseline for background immunofluorescence is set at 0.04% (not depicted). All conditions were performed in duplicate. All data represented as mean \pm SD (error bars). hu-mAb, human monoclonal antibody; IC_{50} , half maximal inhibitory concentration.

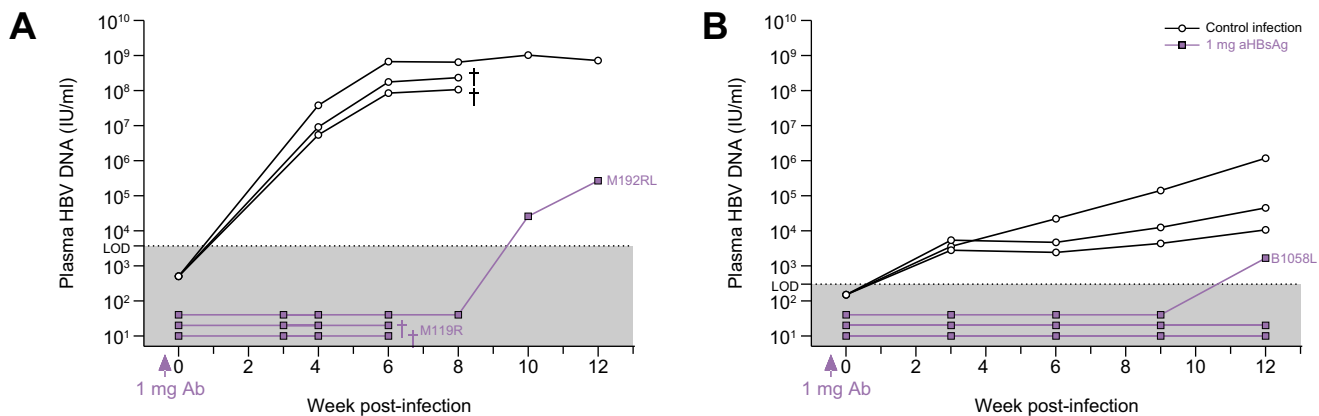


Fig. 3. In vivo prevention of HBV infection. Mice transplanted with hepatocytes from donor C342 (A) or donor HH223 (B) were passively immunized with 1 mg of hu-mAb (IP) 3 days prior to HBV challenge (each $n = 3$). Control mice (each $n = 3$) were infected with the same viral inoculum (patient serum, 10^6 IU/mouse), but without prior passive immunization. HBV DNA was quantified using either the RealStar[®] qPCR (Altona Diagnostics, Germany; LOD = 3,750 IU/ml) (A) or the RealTime m2000 HBV assay (Abbot, US; LOD = 300 IU/ml) (B). †: mouse found dead. hu-mAb, human monoclonal antibody; IP, intraperitoneally; LOD, limit of detection.

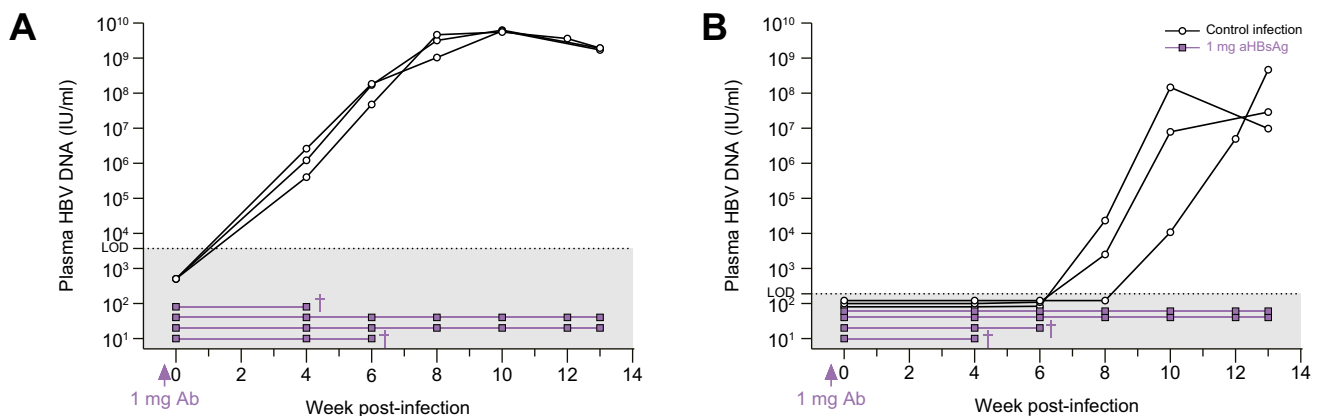


Fig. 4. In vivo prevention of HBV/HDV coinfection. Passive immunization using 1 mg of hu-mAb (IP) was performed 3 days prior to HBV/HDV inoculation ($n = 4$) in mice engrafted with donor C342 hepatocytes. Control mice ($n = 3$) were accordingly infected with cell culture-derived virus (5×10^6 IU HBV and 2.28×10^6 IU HDV per mouse). HBV DNA (A) and HDV RNA (B) was analyzed in mouse plasma via RealStar[®] (reverse transcription)-qPCR (Altona Diagnostics, Germany; LOD HBV = 3,750 IU/ml; LOD HDV = 187.5 IU/ml). †: mouse found dead. hu-mAb, human monoclonal antibody; IP, intraperitoneally; LOD, limit of detection.

increasing levels from week 4 onwards (Fig. 3A). This was also confirmed in mice repopulated with donor HH223 hepatocytes: two out of three passively immunized mice remained HBV DNA negative until at least week 12 post-infection (end of the observation period), one mouse only showed signs of infection at week 12 and all three control mice demonstrated increasing HBV DNA levels from week 3 onwards (Fig. 3B). In the two passively immunized mice that rebounded at week 10 and 12 post-infection, circulating plasma anti-HBsAg was no longer detectable as of week 6 (Fig. S1).

In vivo prevention of HBV/HDV coinfection

Four human-liver chimeric mice were injected with 1 mg of hu-mAb (IP), 3 days prior to inoculation of a viral preparation containing 5×10^6 IU HBV and 2.28×10^6 IU HDV (both cell culture produced). Control mice ($n = 3$), injected with the same virus

prep without prior passive immunization, experienced increasing plasma levels of HBV DNA (Fig. 4A) and HDV RNA (Fig. 4B) from week 4 and 8 onwards, respectively. Interestingly, all passively immunized mice did not show any signs of infection until 13 weeks post-infection when the experiment was terminated. Anti-HBs antibody levels exceeded 1,000 mIU/ml during the first 2-4 weeks following passive immunization, gradually declined over time and became undetectable in our assay at around 8-10 weeks post antibody injection (Fig. S2).

In vivo prevention of HDV superinfection

To evaluate whether a single antibody injection could avert an HDV infection in mice with pre-existing HBV infection, human-liver chimeric mice engrafted with donor L191501 hepatocytes ($n = 7$) were first infected with HBV and 6 weeks later, five out of seven mice were passively immunized with the antibody 3 days

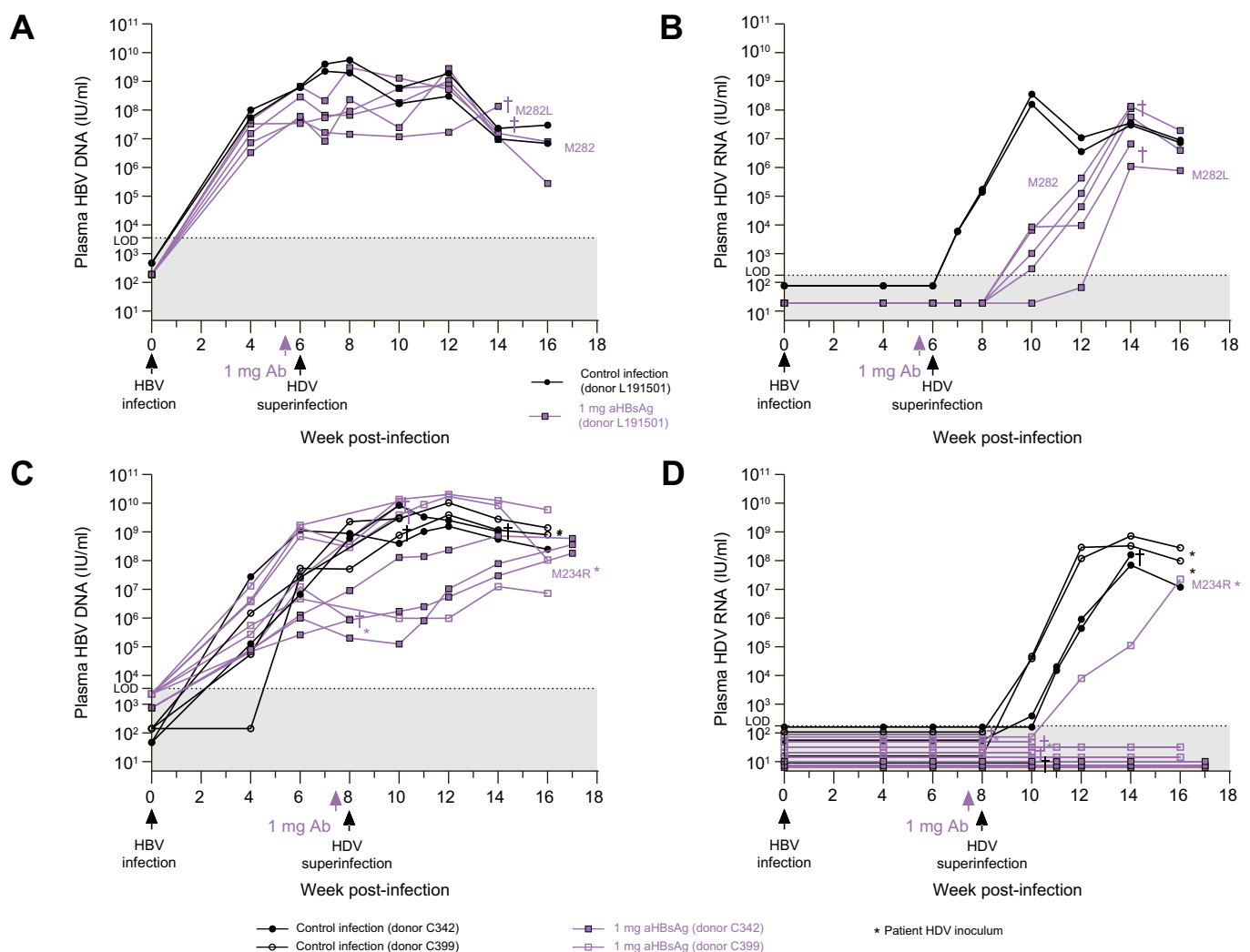


Fig. 5. In vivo prevention of HDV superinfection. (A-B) Mice were engrafted with human hepatocytes from donor L191501 and infected at week 0 with patient-derived HBV (10^6 IU/mouse, $n = 7$). Three days prior to HDV superinfection with patient-derived inoculum (2.55×10^5 IU/mouse) at week 6, passive immunization in 5/7 mice was performed with 1 mg of hu-mAb (IP). (C-D) Mice were engrafted with human hepatocytes from donor C342 ($n = 5$) or C399 ($n = 9$) as depicted and infected at week 0 with cell culture-derived HBV (5×10^6 IU/mouse). Three days prior to HDV superinfection with either cell culture-derived inoculum or patient-derived inoculum (*) at week 8 (both using 2.55×10^5 IU/mouse), passive immunization in 9 ($n = 3$ for C342 mice and $n = 6$ for C399 mice) out of 14 mice was performed with 1 mg of hu-mAb (IP). HBV DNA (A, C) and HDV RNA (B, D) was quantified by RealStar[®] (reverse transcription-)qPCR (Altona Diagnostics, Germany; LOD HBV = 3,750 IU/ml; LOD HDV = 187.5 IU/ml). †: mouse found dead. hu-mAb, human monoclonal antibody; IP, intraperitoneally; LOD, limit of detection.

prior to HDV superinfection (Fig. 5A-B). At the moment of HDV inoculation (week 6), the HBV DNA plateau had been reached in all mice (Fig. 5A). In both control mice, HDV RNA levels could already be detected 1 week after HDV inoculation and these increased steeply to 10^8 IU/ml at week 10. In most passively immunized mice, HDV RNA was only detected at week 10 with a delayed peak at week 14 (Fig. 5B). Accordingly, circulating anti-HBs was lost between week 8 and 14 for all passively immunized mice (Fig. 53A). This experimental setup was repeated in 14 mice engrafted with either donor C342 or C399 hepatocytes, but here, passive immunization (in 9/14 mice) and HDV superinfection was performed at week 8 (Fig. 5C-D). Interestingly, eight mice were completely protected from HDV superinfection and the five animals that reached the endpoint of the observation period (week 16-17) were still HDV RNA negative (Fig. 5D). The single passively immunized mouse that became HDV RNA positive at week 12 also lost its circulating anti-HBs at week 9 (Fig. 53B).

Treatment of chronic HBV infection

Humanized mice with an established HBV infection were treated twice a week for 4 consecutive weeks with 1 mg of hu-mAb (IP; $n = 4$). While non-treated HBV-infected controls ($n = 3$) demonstrated quite stable or moderately increasing plasma HBV DNA levels, all antibody-treated mice showed strongly, although

not statistically significantly, decreasing viral levels on therapy (Fig. 6A-B and Table S1). Specifically, already after 1 week of therapy, mean plasma HBV DNA levels were reduced by $1.78 \times \log_{10}$ compared to non-treated mice. HBV DNA even became undetectable in our assay in one mouse (M240L). On week 2, 3 and 4 (end) of treatment, we observed a $1.55 \times \log_{10}$, $2.09 \times \log_{10}$ and $2.02 \times \log_{10}$ fold reduction, respectively, compared to the controls. Importantly, only one (M257RR) out of four mice rebounded after treatment cessation and this coincided with a complete loss of circulating anti-HBs antibodies (Fig. 6C). Three weeks post-therapy, a mean difference of $2.03 \times \log_{10}$ was still present between the two groups.

Treatment of HBV/HDV-coinfected mice

Six weeks post HBV infection, 13 humanized mice were inoculated with HDV to obtain HBV/HDV-coinfected mice. Four weeks later, the animals were divided in two groups: the first group ($n = 7$) was treated with hu-mAb for 4 weeks, while the second group ($n = 6$) served as untreated controls. At the start of therapy, plasma HBV DNA levels ranged between 10^6 and 10^9 IU/ml, while HDV RNA levels were more diverse, varying from just above the limit of detection (LOD) to almost 10^8 IU/ml, with one mouse (M264RL) having undetectable HDV RNA in our assay (Fig. 7A,C). Two weeks following the onset of therapy, mean HBV DNA and HDV RNA levels

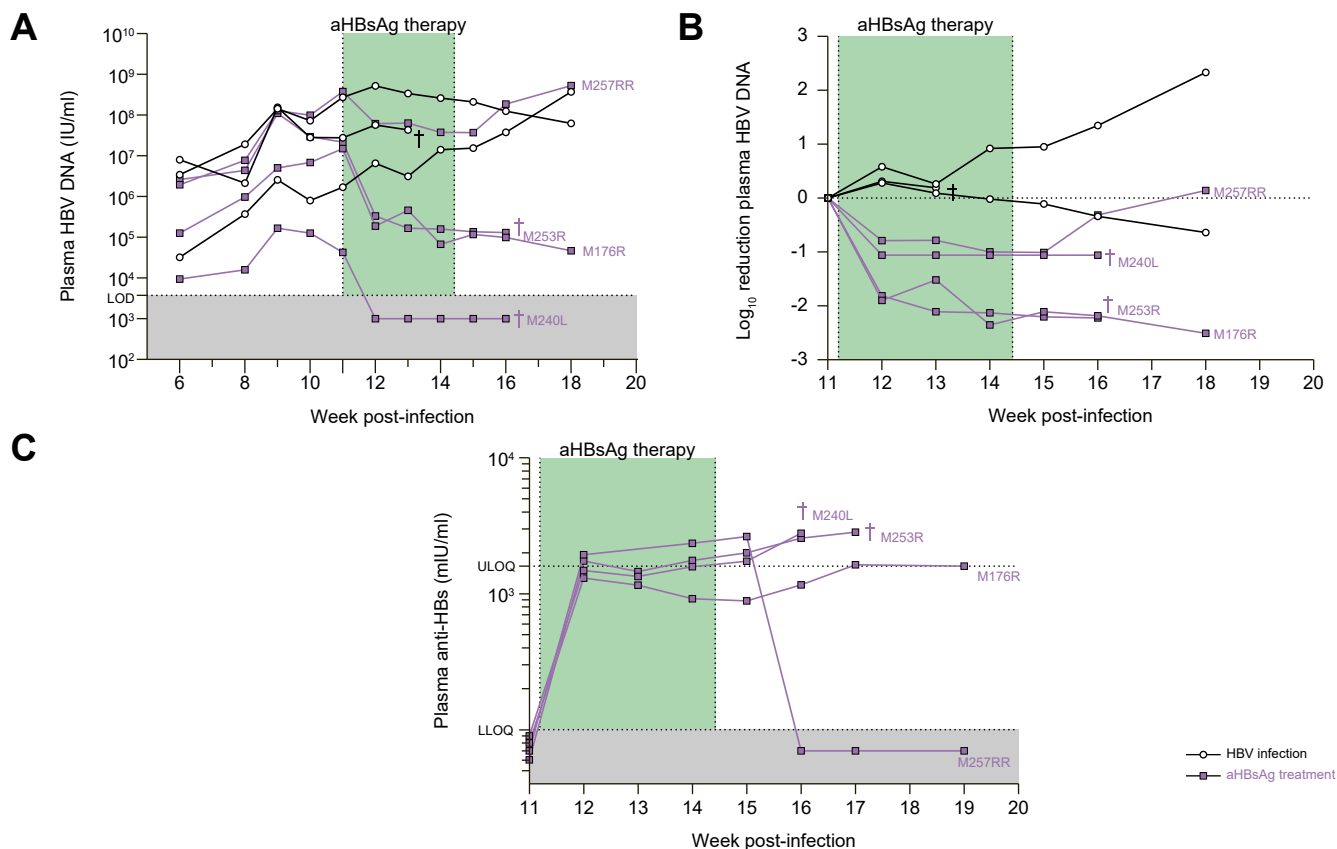


Fig. 6. Treatment of chronic HBV infection. Human-liver chimeric mice (engrafted with donor C342 hepatocytes) were infected with HBV for 11 weeks and then treated with hu-mAb for 4 consecutive weeks: 2 IP injections of 1 mg per week ($n = 4$). Control mice were accordingly infected with cell culture-derived virus (5×10^6 IU HBV/mouse) but were not treated ($n = 3$). (A) Plasma HBV DNA. LOD (HBV) = 3,750 IU/ml. (B) Log₁₀ reduction of plasma HBV DNA, calculated towards levels observed 1 day prior to first antibody injection (week 11). (C) Anti-HBsAg determined in mouse plasma via quantitative ELISA (Wantai, China). LLOQ (anti-HBsAg) = 100 mIU/ml and ULOQ (anti-HBsAg) = 1,600 mIU/ml. †: mouse found dead. hu-mAb, human monoclonal antibody; IP, intraperitoneally; LLOQ, lower limit of quantification; LOD, limit of detection; ULOQ, upper limit of quantification.

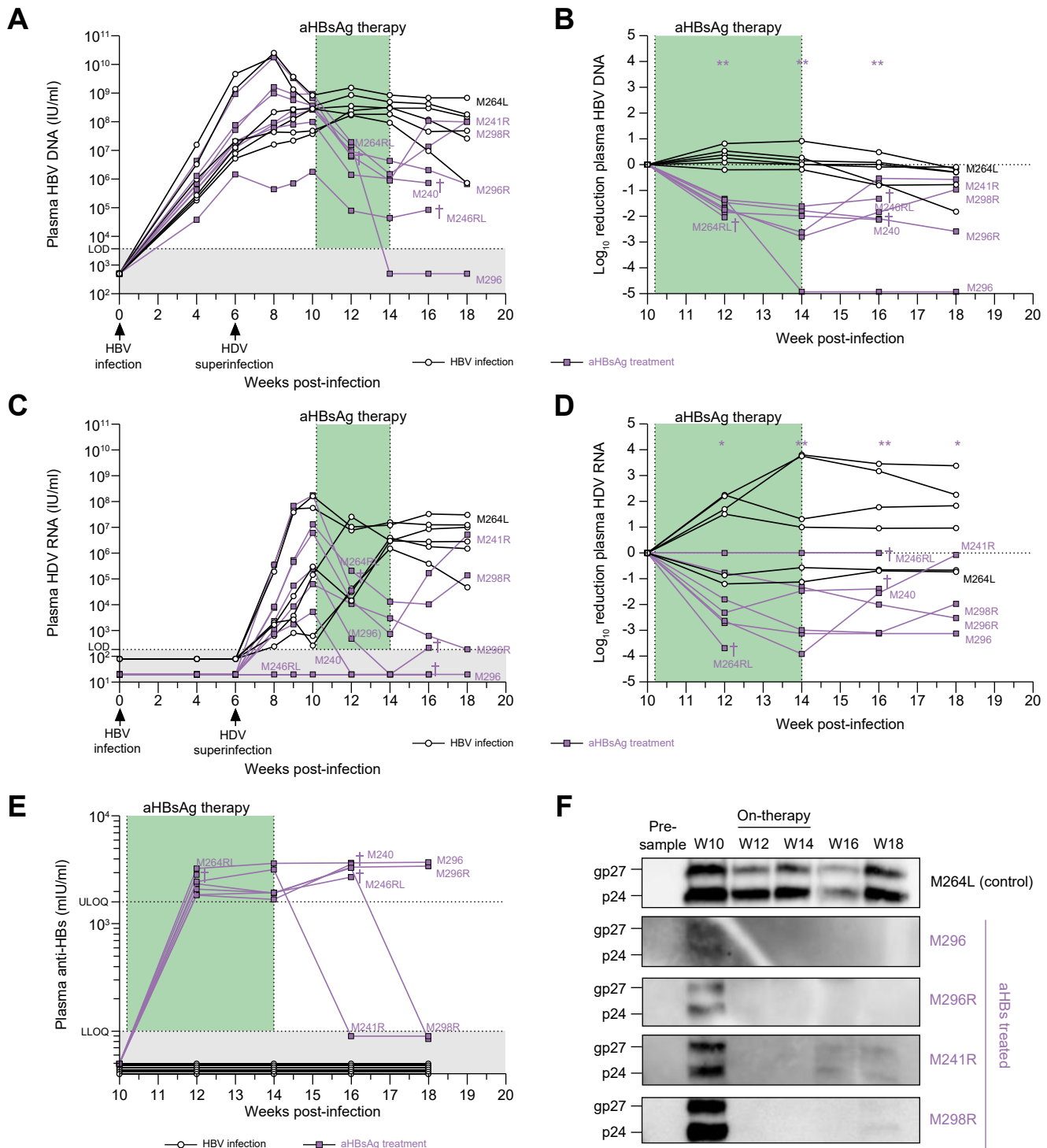


Fig. 7. Treatment of chronic HBV/HDV infection. Human-liver chimeric mice engrafted with donor L191501 hepatocytes were infected with HBV and superinfected with HDV at week 6. From week 10 onwards, mice were treated with hu-mAb for 4 consecutive weeks: 2 IP injections of 1 mg per week ($n = 7$). Control mice were accordingly infected with cell culture-derived virus (5×10^6 IU HBV and 2.55×10^5 IU HDV per mouse) but were not treated ($n = 6$). (A) Plasma HBV DNA. LOD (HBV) = 3,750 IU/ml. (B) Log₁₀ reduction of plasma HBV DNA, calculated towards levels observed 1 day prior to first antibody injection (week 10). (C) Plasma HDV RNA. LOD (HDV) = 187.5 IU/ml. (D) Log₁₀ reduction of plasma HDV RNA, calculated towards levels observed 1 day prior to first antibody injection (week 10). Statistical analyses performed using Mann-Whitney U test: * p value <0.05; ** p value <0.01. (E) Anti-HBsAg determined in mouse plasma via quantitative ELISA. LLOQ (anti-HBsAg) = 100 mIU/ml and ULOQ (anti-HBsAg) = 1,600 mIU/ml. (F) HBsAg Western blot analysis. †: mouse found dead. (g)p, (glycosylated) protein; hu-mAb, human monoclonal antibody; IP, intraperitoneally; LLOQ, lower limit of quantification; LOD, limit of detection; ULOQ, upper limit of quantification.

were significantly reduced compared to controls: $1.96 \times \log_{10}$ ($p < 0.01$) and $2.82 \times \log_{10}$ ($p < 0.05$) difference, respectively (Fig. 7A-D and Table S2). Even more pronounced mean differences could be observed after 4 weeks (end of therapy): $-2.82 \times \log_{10}$ and $-3.15 \times \log_{10}$ (both $p < 0.01$) for HBV DNA and HDV RNA, respectively, while stable or even increasing viral loads were measured in the controls. Two and 4 weeks after treatment cessation, mean differences of $-1.97 \times \log_{10}$ and $-1.69 \times \log_{10}$ for HBV DNA, respectively, and $-3.20 \times \log_{10}$ and $-3.10 \times \log_{10}$ for HDV RNA, respectively, were observed; these differences were accompanied by hu-mAb plasma levels exceeding 1,000 mIU/ml in treated animals. However, two mice (M241R and M298R) demonstrated HBV/HDV rebound at week 2-4 post-therapy, which coincided with hu-mAb loss at these time points (Fig. 7E). Remarkably, HBsAg was observed at the onset of therapy in the treated mice (week 10) but was undetectable on therapy (based on western blot analysis) (Fig. 7F). For the two mice showing strongly diminished or even absent viral loads post-therapy (M296 and M296R), HBsAg was still absent. On the other hand, the post-therapy viral rebound observed in M241R (at week 16) and M298R (at week 18) was accompanied by recurrence of HBsAg.

Discussion

The excessive HBsAg release in patients with chronic HBV tolerizes antibody- and cell-mediated immune responses, representing a major obstacle to viral eradication with current treatments.^{25,26} Consequently, it is of utmost importance to find ways to break immune tolerance, enabling the host to mount effective immune responses to clear the virus and to prevent HDV superinfection. Herein, we demonstrate the prophylactic and therapeutic efficacy of a hu-mAb targeting the HBV envelope in the context of HBV and HDV infection *in vitro* and in human-liver chimeric mice.

The hu-mAb neutralized HBV and HDV *in vitro* with respective IC_{50} values of 3.53 ng/ml (~ 0.23 nM) and 3.58 ng/ml (~ 0.24 nM) and can be considered as highly potent, since Hehle *et al.*²⁷ evaluated 170 different hu-mAbs isolated from natural HBV controllers or vaccinated individuals and showed that only 35% of antibodies harbored IC_{50} values < 50 ng/ml.

Next, we demonstrated efficient prevention of HBV mono- and HBV/HDV coinfection in human-liver chimeric mice. Conceivably, once a chronic HBV infection is fully established, preventing HDV superinfection may be even more challenging due to the presence of large quantities of HBsAg. However, depending on the hepatocyte donor, we showed either near complete prevention (donor C342 and C399) or at least delayed HDV RNA levels (donor L191501). Notably, transplantation of mice with donor L191501 human hepatocytes rendered the animals more prone to hepatitis infection compared to other donors tested (*data not shown*), which may explain viral breakthrough. Overall, our HDV superinfection prevention data is quite impressive since only a single injection of 1 mg hu-mAb was sufficient to overcome the high load of circulating HBsAg and to (partially) neutralize the high-titer HDV inoculum. Furthermore, since HDV can silently persist and replicate in human hepatocytes for up to 6 weeks, the hu-mAb effectively prohibits entry of HDV virions into hepatocytes.^{28,29}

Notably, antibody half-life varies throughout our experiments, *i.e.* the antibody is still present in circulation 3-4 weeks or longer after a 1 mg injection. This may potentially be explained by variability in body weight (and hence distribution volume) since we injected 1 mg of antibody irrespective of mouse body

weight. Furthermore, antibody clearance might be greatly accelerated by high replication of the virus (high circulating viral load). The source of viral inoculum also appears to influence the success of prevention and lack of viral rebound. For example, in the prophylactic HBV/HDV coinfection experiment, cell culture-derived virus was used and no viral rebound was observed, while in the prophylactic HBV mono-infection experiment, patient-derived virus was applied and HBV DNA rebound was observed. Additionally, more animals were protected from HDV superinfection when infected with cell culture-derived viruses. Therefore, it seems that primary patient-derived virus preparations are intrinsically less sensitive to antibody neutralization, or may contain variants that are.

The therapeutic potential of the hu-mAb was furthermore demonstrated by (significantly) reduced mean viral plasma levels at the end of a 4-week treatment in HBV-infected ($2.02 \times \log_{10}$ drop) and HBV/HDV-coinfected mice ($2.82 \times \log_{10}$ and $3.50 \times \log_{10}$ drops for HBV and HDV, respectively). Since the repeated injections of antibody result in a clear drop in HBV DNA (and HDV RNA levels) in this therapeutic setup, we assume that the single dose regimen in the HDV superinfection prevention experiment was insufficient to show an antiviral effect on the already established HBV infection (*i.e.* no effect on HBV DNA). Interestingly, our 4-week treatment data are in line with the most potent hu-mAb (namely Bc1.187), which was tested by Hehle *et al.*²⁷ in HUHEP mice (weekly ~ 1 mg (50 mg/kg) hu-mAb IP for 3 weeks), and induced a $1.76 \times \log_{10}$ decrease in serum HBV DNA at the end of therapy. Notably, circulating HBsAg levels dropped and remained undetectable for 2 weeks following the last injection in 60% of treated animals. Importantly, these mice had lower initial HBV DNA levels ($< 10^6$ IU/ml) compared to mice in our study. Another hu-mAb (HBC34) was evaluated at a very comparable schedule, but at lower dose (a 4-week treatment with 2 IP injections per week of 1 mg/kg) in HBV/HDV-coinfected USB/USG mice.^{30,31} They revealed a similar reduction in HBV DNA and HDV RNA levels (mean 1 to $2.7 \times \log_{10}$ and 2.4 to $2.5 \times \log_{10}$ reduction, respectively, in both models) and a concomitant 2.7 to $2.8 \times \log_{10}$ reduction of HBsAg levels after 4 weeks. Finally, we also show on-treatment HBsAg loss with rebound after treatment cessation, a phenomenon that is frequently noted upon withdrawal of mAb therapy.^{27,32} Interestingly, the HBsAg loss might provide a therapeutic window for viral eradication using NAs, possibly in combination with other antivirals, that could take advantage of a (partially) reconstituted adaptive immune response.^{23,24} Interestingly, although no effect on HBsAg was seen when bulevirtide was used as monotherapy, HBsAg decreases could be observed when combined with peg-IFN α .^{17,20} Notably, decreasing HBsAg might be a valuable therapeutic strategy, but currently no direct evidence is available to support this hypothesis and therefore, additional studies addressing the relationship between circulating HBsAg levels and HBV-specific immune responses are required to provide further insights on the immunobiology of HBV; presumably, additional immune activation would be necessary to control the virus.³³ Nowadays, polyclonal antibodies derived from pooled plasma from recovered or vaccinated individuals (hepatitis B immunoglobulin) are successfully and routinely used in clinical practice as post-exposure prophylaxis, to avoid HBV recurrence after liver-transplantation or therapeutic immunosuppression, or to prevent perinatal transmission.³⁴⁻³⁶ However, only a small antibody proportion in hepatitis B immunoglobulin preparations is actually HBV-specific and neutralizing, and blood products always

remain subject to bio-safety concerns, especially for immunosuppressed patients.^{37,38} Consequently, monoclonal antibodies may be an attractive alternative since they represent a stable, reproducible source for prolonged immunotherapy. Hu-mAbs have already been successfully explored in many therapeutic fields such as oncology,³⁹ auto-immune diseases⁴⁰ and recently also for infectious diseases such as SARS-CoV-2.^{41,42} Our research group also demonstrated successful hu-mAb use in the context of HCV infection.^{43–45} Regarding other HBV-specific hu-mAbs, previous studies have similarly shown efficient prevention, delayed infection, or therapeutic efficacy in cell culture,^{27,46–48} chimpanzees^{49–54} and various mouse models.^{27,38,54–58} Some have reached clinical stages as well, such as lenvirmab⁵⁹ and VIR-3434, the Fc-engineered version of antibody HBC34, with extended serum half-life; NCT04423393 and NCT04856085.⁶⁰ Hu-mAb studies involving HDV prophylaxis or therapy are currently very limited: Li *et al.*⁴⁰ demonstrated prevention in a mouse model harboring human NTCP receptors in the absence of HBV infection and, moreover, on a mouse background; and HBC34 led to a potent decrease in HBV/HDV viremia in HBV/HDV-coinfected USB/USG mice.^{30,31}

Currently evaluated or approved antibody dosages vary tremendously in human applications, even beyond the field of infectious diseases. For example, omalizumab, a humanized recombinant monoclonal anti-IgE antibody approved for the treatment of allergic asthma is recommended at 75 to 600 mg every 2 weeks.⁶¹ Rituximab, a genetically engineered chimeric mouse/human mAb approved for rheumatoid arthritis is recommended at two infusions of 1,000 mg IV every 2 weeks.⁶² Eculizumab, a humanized mAb approved for myasthenia gravis, is administered at 600 mg IV weekly for 4 weeks, followed by 900 mg IV in the 5th week and then, 900 mg every 2 weeks as maintenance therapy.⁶³ Finally, the FDA recently authorized two mAbs (casirivimab and imdevimab) for the treatment of COVID-19 disease at dosages as high as 1,400 mg IV.^{64,65} These examples illustrate the high variability of applied doses in various applications, but also that much higher dosages are achievable and tolerated compared to our regimen. Assuming an average mouse body weight of 15 g, we applied about 66.7 mg/kg per injection. In the treatment regimen, we hence administered a total dose of 533 mg over a 4-week period; clearly in range of what is feasible in humans. However, dosages used in mice cannot simply be translated into human doses. More specifically, the common perception of scaling of dose based on body weight (mg/kg) alone is not entirely correct. This is

primarily because the functional systems in species vary which, in turn, alters pharmacokinetics.⁶⁶

The main putative mechanism of action of the HBsAg-targeting antibody in the applied mouse model is functional neutralization of the viral envelope, which directly blocks entry of HBV and HDV, and consequently prevents spread of infection to naïve hepatocytes. Furthermore, the antibody also promotes HBsAg reduction (or clearance) in the circulation. It has also been shown that HBsAg-specific antibodies could be internalized into infected cells and therefore partially inhibit HBsAg secretion from these cells, implicating intracellular blocking of virion and protein release as an additional mechanism of action.^{67,68} Importantly, in the context of infected patients, additional intrinsic effector functions could be induced through the formation of immune complexes. More specifically, clearance of circulating virions, antigens or infected cells could be promoted via antibody-dependent cell-mediated cytotoxicity and/or other Fc-mediated effector responses such as complement lysis or phagocytosis.^{55,56,69–73} Accordingly, many therapeutic advantages could be proposed in a clinical setting: 1) reduction of the hepatic accumulation of covalently closed circular DNA, one of the main causes of HBV persistence;^{74,75} 2) mediation of serum HBV/HDV and HBsAg clearance, which may ultimately counteract the challenging T-cell exhaustion mechanisms that are characteristic in patients with chronic HBV infection;⁷⁶ 3) overall risk reduction of viral reactivation (following liver-transplantation);⁷⁷ and finally 4) potential allowance of safe withdrawal of antiviral drugs.⁷⁸

In conclusion, we show the added value of an entry-inhibiting hu-mAb in the context of HBV and HDV (super)infection prophylaxis and its potential use as treatment for chronic HBV or HBV/HDV (co-)infection. For the experiments presented herein, we used either patient-derived virus or cell culture-derived virus (genotype D for HBV and genotype 1 for HDV) in human-liver chimeric mice that were transplanted with hepatocytes from four different donors. Further confirmation using additional viral genotypes and various hepatocyte donors with distinct genetic backgrounds is warranted. Overall, our data suggest that this hu-mAb is an interesting candidate to complement current therapies or antivirals (under development) to eradicate both HBV and HDV infections. Decreasing or even eliminating the high levels of circulating HBsAg in patients with chronic infection may provide an opportunistic window for other antiviral therapies targeting later steps in the viral life cycle or the immune system to eventually cure chronic infection.

Abbreviations

hu-mAb, human monoclonal antibody; HBsAg, hepatitis B surface antigen; IC₅₀, half maximal inhibitory concentration; IP, intraperitoneally; LLOQ, lower limit of quantification; LOD, limit of detection; NTCP, sodium taurocholate co-transporting polypeptide.

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Conflict of interest

Nothing to declare related to the content of this manuscript.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

PM devised the study. General experimental setup was designed by PM and RB. RB and FVH performed *in vitro* neutralization studies. Animal work was conducted by RB, LV, AAM and PM. Viral parameters were monitored in mouse plasma by RB and FVH. GLR provided the antibody clone. SC, HW and PR delivered patient-derived viruses that were essential for the execution of the study. Data analyses and writing were performed by RB and PM. All authors approved the final version of the manuscript.

Data availability statement

The data that supports the findings of this study is available upon request. Please contact the corresponding author.

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Supplementary data

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Supplemental information

A human monoclonal antibody against HBsAg for the prevention and treatment of chronic HBV and HDV infection

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Supplementary materials and methods

Cells

HepAD38, Huh7.5, PLC/PRF/5, HepG2.hNTCP, Huh7.5.hNTCP and HepNB2.7 cells were all cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum, 1% non-essential amino acids, 1% L-glutamine and antibiotics, unless otherwise specified.

Production of HBV/HDV inocula

The HBV inoculum (genotype D) was produced using the stable tetracycline-regulated HepAD38 cell line as previously described [2]. In brief, tetracycline (1 µg/ml) was supplemented into the culture medium for one week, cells were washed with PBS and further cultured in medium without tetracycline. Three days later, virus-containing supernatant was collected every three to four days and for three consecutive weeks. For the production of HDV (genotype 1), either Huh7.5 cells were co-transfected with plasmids pSVL(D)3 and pT7HB2.7, or alternatively, PLC/PRF/5 cells were transfected with pSVL(D)3, both using TransIT LT1-reagent (Mirus Bio LLC, REF-2300, USA). Virus containing supernatant was collected every two to three days for two weeks. The HBV and HDV supernatants were sterile filtered (0.45 µm) and concentrated using the PEG Virus Precipitation kit (Abcam, REF-ab102538, UK). Finally, the viral load was determined by commercial RealStar® HBV or HDV (RT-)qPCR (Altona Diagnostics, Germany) after automated nucleic acid extraction (NucliSENS® EasyMAG®, Biomérieux, France).

In vitro prevention of HBV and HDV infection and immunofluorescent staining

For *in vitro* prevention of HBV, HepG2.hNTCP cells were plated (day 0) at a density of 20,000 cells/well in Poly-L-Lysine coated (Sigma Aldrich, REF-P9155) 96-well plates (Greiner CELLSTAR, REF-655090). One day later, anti-HBsAg was added in duplicate at 5-fold serial

dilutions ranging from 10 µg/ml (666.67 nM) to 0.128 ng/ml (8.53 pM). Two hours later, HBV inoculum (4,990 IU/cell) in culture medium supplemented with 4% PEG and 2.5% DMSO was applied. After 24 hours of virus incubation (day 2), cells were washed three times with PBS to remove the virus and further cultured in medium supplemented with 2.5% DMSO and the respective concentration of anti-HBsAg. day 8, infection was assessed using immunofluorescent (IF) staining of HBV-core positive cells. Therefore, cells were washed three times with PBS, fixed (4% paraformaldehyde, 20 min.), permeabilized (Nonidet® P40-Substitute, 15 min.), blocked (PBS containing 2% BSA and 10% goat serum, 30 min.) and stained using a polyclonal rabbit anti-HB-Core antibody (38 µg/ml) for 1 hour (DakoCytomation, REF-B0586), followed by 1 hour incubation of an AlexaFluor (AF)-488 labeled goat anti-rabbit IgG secondary antibody at 2 µg/ml (Invitrogen, REF-A11034) and DAPI nuclear staining at 1.42 µl/ml (MolProbes, REF-D3571). DAPI and AF-488 signals were detected using automated spinning disk microscopy using a 40X objective (CSU-X1, Nikon). Per condition, a 20x10 field was captured (in duplicate) and positive cells were automatically counted using ImageJ software v1.53c.

In vitro prevention of HDV infection was similarly conducted with some slight modifications to the above described procedure. First, The hu-mAb was separately incubated with the HDV inoculum (4 IU/cell) for 1 hour prior to addition onto HepNB2.7, HepG2.hNTCP and Huh7.5.hNTCP cells. For IF staining, blocking was performed using 5% skim milk in PBS. As primary antibody, we used ,000 diluted EDTA-plasma from a HDV-cleared patient harboring undetectable HBV DNA and HDV RNA levels at the time of blood donation. Cells were overnight incubated at 4°C. An AF-488 labeled goat anti-human secondary antibody at 2 µg/ml (Invitrogen, REF-A-11013) was then applied for 1 hour. Imaging was performed using the Leica TCS-SPE with 20X objective. Per condition, 3 random pictures were taken (in duplicate).

SDS-PAGE and Western blot analysis

Mouse plasma samples (exactly 0.5µl for all samples) were denatured in the presence of the Bolt™ LDS sample buffer and the Bolt™ Reducing agent (Thermo Fisher Scientific) at 70°C for 10 minutes. After denaturation, samples were submitted to SDS-PAGE gel electrophoresis on a 12% acrylamide gel, using the Novex® mini gel Tank (Thermo Fisher Scientific). Afterwards, proteins were transferred to a polyvinylidene difluoride membrane (0.2 µm pore size; Thermo Fisher Scientific), using the Novex® Blot module (Thermo Fisher Scientific). Blocking of unsaturated sites was performed with 5% (w/v) skim milk powder in Tris-buffered saline supplemented with 0.1% (v/v) Tween-20 (TBS-T) for 2h at room temperature. The membranes were incubated overnight at 4°C with goat anti-HBsAg antibody (70-HG15; Fitzgerald industries; 1/1,000) diluted in blocking buffer. Membranes were washed with TBS-T and subsequently incubated for 2h at room temperature with horse-radish peroxidase-conjugated rabbit anti-goat antibody (31402; Thermo Fisher Scientific; 1/20,000) diluted in blocking buffer. Immunoblots were developed using the SuperSignal™ West Femto Maximum Sensitivity Substrate kit (Thermo Fisher Scientific) and exposed to the ImageQuant LAS4000 chemiluminescent imaging system (GE Healthcare, Diegem, Belgium).

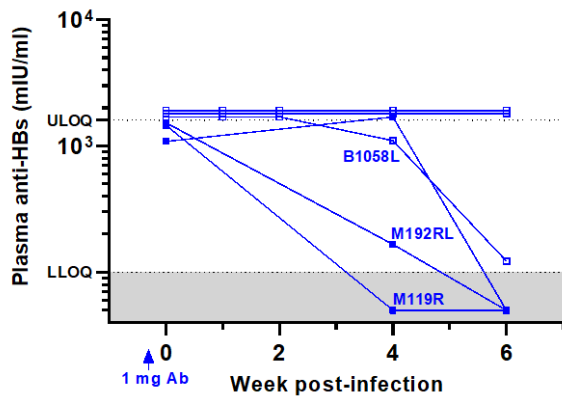


Fig. S1: In vivo prevention of HBV infection. Mice transplanted with hepatocytes from donor C342 (full squares, n=3) or donor HH223 (empty squares, n=3) were passively immunized with 1 mg of hu-mAb (IP) 3 days prior to HBV challenge at week 0 with patient serum (10^6 IU/mouse). Plasma anti-HBsAg levels were determined using quantitative ELISA (Wantai, China) with LLOQ = 100 mIU/ml and ULOQ = 1,600 mIU/ml. All plasma samples collected 1 week prior antibody treatment were tested negative for anti-HBsAg (not depicted).

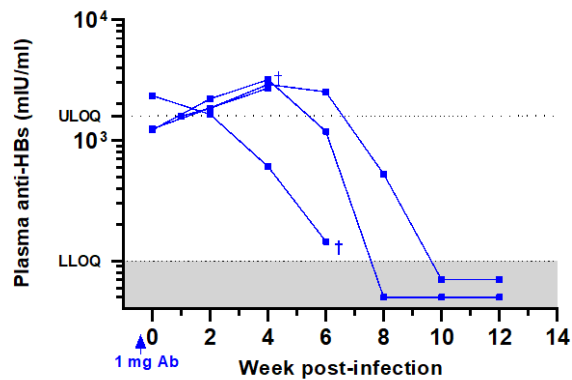


Fig. S2: In vivo prevention of HBV/HDV co-infection. Passive immunization using 1 mg of hu-mAb (IP) was performed 3 days prior to HBV/HDV inoculation in mice engrafted with donor C342 hepatocytes (n=4). Cell-culture derived virus (5×10^6 IU HBV and 2.28×10^6 IU HDV per mouse) was used as viral inoculum. Plasma anti-HBsAg levels were determined using quantitative ELISA (Wantai, China) with LLOQ = 100 mIU/ml and ULOQ = 1,600 mIU/ml. All plasma samples collected 1 week prior antibody treatment were tested negative for anti-HBsAg (not depicted). †: mouse found dead.

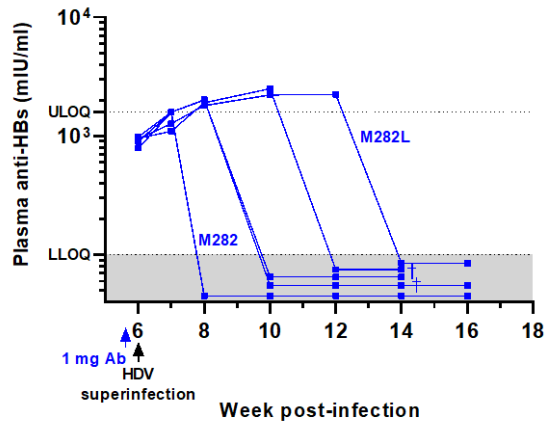
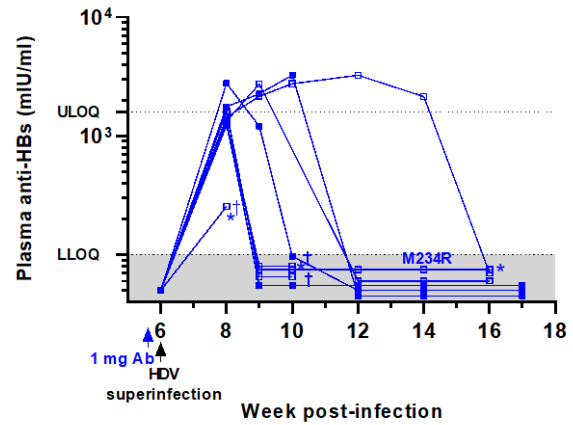
A.**B.**

Fig. S3: In vivo prevention of HDV superinfection. (A) Mice engrafted with hepatocytes from donor L191501 were infected at week 0 with patient-derived HBV (10^6 IU/mouse, $n=7$). Three days prior to HDV superinfection with patient-derived inoculum (2.55×10^5 IU/mouse) at week 6, passive immunization in 5 out of 7 mice was performed with 1 mg of hu-mAb (IP). (B) Mice engrafted with hepatocytes from donor C342 ($n=5$, full squares) or C399 ($n=9$, empty squares) were infected at week 0 with cell culture-derived HBV (5×10^6 IU/mouse). Three days prior to HDV superinfection with cell culture-derived inoculum (2.55×10^5 IU/mouse) at week 8, passive immunization in 9 ($n=3$ for C342 mice; and $n=6$ for C399 mice) out of 14 mice was performed with 1 mg of hu-mAb (IP). Plasma anti-HBsAg levels were determined using quantitative ELISA (Wantai, China) with LLOQ = 100 mIU/ml and ULOQ = 1,600 mIU/ml. All plasma samples collected 1 week prior antibody treatment were tested negative for anti-HBsAg (not depicted). *: patient HDV inoculum, instead of cell culture derived preps, was used for HDV superinfection. †: mouse found dead.

Table S1: Treatment of chronic HBV infection.

	Mean HBV DNA levels (IU/ml)		Mean Log ₁₀ Reduction			Mann-Whitney U- test	
	Control	Treated	Control	Treated	Δ Means \pm SEM	P-value	Sign. (P < 0.05)
0w of therapy (baseline)	1.01E+08 (n=3)	1.04E+08 (n=4)					
1w of therapy	1.96E+08 (n=3)	1.56E+07 (n=4)	0.39 (n=3)	-1.39 (n=4)	-1.78 \pm 0.33	0.05714	N.s.
2w of therapy	1.28E+08 (n=3)	1.61E+07 (n=4)	0.18 (n=3)	-1.37 (n=4)	-1.55 \pm 0.35	0.05714	N.s.
3w of therapy	1.37E+08 (n=2)	9.56E+06 (n=4)	0.45 (n=2)	-1.64 (n=4)	-2.09 \pm 0.60	0.1333	N.s.
4w of therapy (end of therapy)	1.13E+08 (n=2)	9.41E+06 (n=4)	0.42 (n=2)	-1.59 (n=4)	-2.02 \pm 0.58	0.1333	N.s.
1w post-therapy cessation	8.16E+07 (n=2)	4.63E+07 (n=4)	0.50 (n=2)	-1.44 (n=4)	-1.95 \pm 0.87	0.2667	N.s.
3w post-therapy cessation	2.16E+08 (n=2)	2.64E+08 (n=2)	0.85 (n=2)	-1.18 (n=2)	-2.03 \pm 1.99	0.6667	N.s.

Human-liver chimeric mice (hepatocyte donor C342 engrafted, n=7) were chronically infected with HBV for 11 weeks and 4 mice out of these were treated with HBsAg antibody for 4 consecutive weeks: 2 IP injections of 1 mg per week. Control mice (n=3) were infected in the same way with cell-culture derived virus (5×10^6 IU HBV per mouse), but were non-treated. Mean plasma HBV DNA levels in mouse plasma are shown at indicated time points. The mean log₁₀ reduction is represented and calculated towards one day prior to first antibody injection (0w of therapy-baseline). Mice with undetectable HBV DNA levels were imputed as the LOD for HBV DNA detection, i.e. 3,750 IU/ml. Statistics: n.s (non-significant); p-value > 0.05. SEM: standard error of the mean. Δ : difference of mean log₁₀ reduction between treated versus control group. N: number of mice are indicated per time point. W: week.

Table S2: Treatment of chronic HBV/HDV infection.

		Mean viral loads (IU/ml)		Mean Log ₁₀ Reduction			Mann-Whitney U- test	
		Control	Treated	Control	Treated	Δ Means \pm SEM	P-value	Sign. (P < 0.05)
HBV DNA	0w of therapy (baseline)	3.09E+08 (n=6)	3.79E+08 (n=7)					
	2w of therapy	5.59E+08 (n=6)	8.32E+06 (n=7)	0.32 (n=6)	-1.64 (n=7)	-1.96 \pm 0.17	0.001166	Yes, **
	4w of therapy (end of therapy)	3.73E+08 (n=6)	1.31E+06 (n=6)	0.20 (n=6)	-2.62 (n=6)	-2.82 \pm 0.52	0.002165	Yes, **
	2w post-therapy cessation	2.64E+08 (n=6)	2.07E+07 (n=6)	-0.17 (n=6)	-2.14 (n=6)	-1.97 \pm 0.64	0.008658	Yes, **
	4w post-therapy cessation	1.81E+08 (n=6)	5.07E+07 (n=4)	-0.57 (n=6)	-2.26 (n=4)	-1.69 \pm 0.85	0.06667	N.s.
HDV RNA	0w of therapy (baseline)	3.66E+07 (n=6)	2.81E+07 (n=7)					
	2w of therapy	8.99E+06 (n=6)	3.89E+04 (n=7)	0.94 (n=6)	-1.99 (n=7)	-2.92 \pm 0.78	0.01399	Yes, *
	4w of therapy (end of therapy)	6.57E+06 (n=6)	2.94E+03 (n=6)	1.37 (n=6)	-2.13 (n=6)	-3.50 \pm 1.04	0.008658	Yes, **

	2w post-therapy cessation	9.88E+06 (n=6)	3.03E+04 (n=6)	1.34 (n=6)	-1.86 (n=6)	-3.20 ± 0.88	0.008658	Yes, **
	4w post-therapy cessation	9.58E+06 (n=6)	1.34E+06 (n=4)	1.18 (n=6)	-1.92 (n=4)	-3.10 ± 0.99	0.03810	Yes, *

Human-liver chimeric mice (hepatocyte donor L191501 engrafted, n=13) were chronically infected with HBV for 6 weeks and then superinfected with HDV. At week 10, 7 out of 13 mice were treated with HBsAg antibody for 4 consecutive weeks: 2 IP injections of 1 mg per week. Control mice (n=6) were infected in the same way with cell-culture derived virus (5×10^6 IU HBV per mouse and 2.55×10^5 IU HDV per mouse), but were non-treated. Mean HBV DNA and HDV RNA levels in mouse plasma are shown at indicated time points. The mean \log_{10} reduction is represented and calculated towards one day prior to first antibody injection (0w of therapy-baseline). Mice with undetectable viral levels were imputed as the LOD: 3,750 IU/ml (for HBV DNA) and 187.5 IU/ml (for HDV RNA). Statistics: * p-value < 0.05; ** p-value < 0.01; n.s.(non-significant): p-value > 0.05. SEM: standard error of the mean. Δ : difference of mean \log_{10} reduction between treated versus control group. N: number of mice are indicated per time point. W: week.

Supplementary references

1. Depraetere, S., et al., *Human B cell growth and differentiation in the spleen of immunodeficient mice*. Journal of immunology (Baltimore, Md. : 1950), 2001. **166**(5): p. 2929-36.
2. Ladner, S.K., et al., *Inducible expression of human hepatitis B virus (HBV) in stably transfected hepatoblastoma cells: a novel system for screening potential inhibitors of HBV replication*. Antimicrobial agents and chemotherapy, 1997. **41**(8): p. 1715-20.