

1 **The potent broadly neutralizing antibody VIR-3434 controls Hepatitis** 2 **B and D Virus infection and reduces HBsAg in humanized mice**

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43 **Abstract**

44 **Background & Aims**

45 Chronic hepatitis B is a major global public health problem, and coinfection with
46 hepatitis delta virus (HDV) worsens disease outcome. Here, we describe a hepatitis B
47 virus (HBV) surface antigen (HBsAg)-targeting monoclonal antibody (mAb) with the
48 potential to promote functional cure of chronic hepatitis B and D to address this unmet
49 medical need.

50 **Methods**

51 HBsAg-specific mAbs were isolated from memory B cells of HBV vaccinated
52 individuals. In vitro neutralization was determined against HBV and HDV enveloped
53 with HBsAg representing eight HBV genotypes. Human liver-chimeric mice were
54 treated twice weekly with a candidate mAb starting three weeks post HBV inoculation
55 (spreading phase) or during stable HBV or HBV/HDV coinfection (chronic phase).

56 **Results**

57 From a panel of human anti-HBs mAbs, VIR-3434 was selected and engineered for
58 pre-clinical development. VIR-3434 targets a putative conserved, conformational
59 epitope within the antigenic loop of HBsAg and neutralized HBV and HDV infection
60 with >12,000-fold higher potency than Hepatitis B Immunoglobulins in vitro.
61 Neutralization was pan-genotypic against strains representative of HBV genotypes A-
62 H. In the spreading phase of HBV infection in human liver-chimeric mice, a parental
63 mAb of VIR-3434 (HBC34) prevented HBV dissemination and intrahepatic HBV RNA
64 and cccDNA increase. In the chronic phase of HBV infection or co-infection with HDV,
65 HBC34 treatment decreased circulating HBsAg by >1 log and HDV RNA by >2 logs.

66

67 **Conclusions**

68 This in vitro and in vivo characterization identified the potent anti-HBs mAb VIR-3434,
69 which reduces circulating HBsAg and HBV/HDV viremia in human liver-chimeric mice.
70 VIR-3434 is currently in clinical development for treatment of patients with chronic
71 hepatitis B or D.

72

73 **Lay summary**

74 Chronic infection with hepatitis B virus places approximately 290 million individuals
75 worldwide at risk for severe liver disease and cancer. Currently available treatments
76 result in low rates of functional cure or require lifelong therapy that does not eliminate
77 the risk of liver disease. We isolated and characterized a potent, human antibody that
78 neutralizes hepatitis B and D viruses and reduces infection in a mouse model. This
79 antibody could provide a new treatment for patients with chronic hepatitis B and D.

80

81 **Highlights**

- 82 • Identification of a human mAb VIR-3434 that potently neutralizes HBV and HDV
- 83 • VIR-3434 targets a conserved, conformational epitope of the HBsAg antigenic loop
- 84 • VIR-3434 treatment blocks intrahepatic HBV spread in human liver-chimeric mice
- 85 • VIR-3434 treatment reduces circulating HBsAg and HDV RNA in co-infected mice
- 86 • Data have enabled clinical development of VIR-3434 against chronic hepatitis B/D

87

88 Introduction

89 Chronic infection with HBV is one of the leading causes of liver cirrhosis and
90 hepatocellular carcinoma. Despite a widely available and efficacious vaccine against
91 HBV, approximately 290 million people are currently living with chronic Hepatitis B
92 (CHB) resulting in an estimated 820,000 deaths per year [1].

93 Approved treatments for CHB include nucleos(t)ide reverse transcriptase
94 inhibitors (NRTIs) and pegylated-interferon alpha (PEG-IFN α); however, neither
95 treatment results in high rates of HBsAg loss. Therapy with NRTIs leads to elimination
96 of HBV DNA in circulation but has limited effect on HBsAg levels. Long-term NRTI
97 therapy reduces but does not eliminate the risk of hepatocellular carcinoma and
98 requires lifelong treatment for most patients. Treatment with PEG-IFN α can induce
99 HBsAg loss, but only in a small fraction of patients (<10%) and the treatment is
100 generally associated with increased flu-like side effects related to the
101 immunomodulatory effects of the treatment [2]. Additionally, Hepatitis B
102 Immunoglobulins (HBIG), which are polyclonal human anti-HBs antibodies, purified
103 from the serum of vaccinees, have long been used for preventative indications,
104 specifically perinatal mother-to-child HBV transmission and of re-infection after liver
105 transplantation [3]. These limited treatment options underscore the need for novel
106 therapies against CHB that are finite and well tolerated. Effective treatments aim at
107 inducing HBV functional cure, which is defined as sustained suppression of HBV DNA
108 and loss of HBsAg off treatment.

109 HBV is a small, enveloped virus from the *Hepadnaviridae* family, with a relaxed-
110 circular DNA genome packaged into a capsid formed by dimers of the viral core protein.
111 The capsid is surrounded by a lipid bilayer, into which the three HBV surface antigen

112 proteins (HBsAg) – large (L-HBsAg), middle (M-HBsAg) and small (S-HBsAg) – are
113 embedded. All three surface proteins share the same C-terminal S-HBsAg domain [4].
114 HBsAg has multiple functions in the viral replication cycle, including facilitating
115 hepatocyte binding and entry by reversible attachment of HBsAg to heparan sulfate
116 proteoglycans (HSPGs) on the surface of hepatocytes [5], followed by non-reversible,
117 high-affinity interaction of the viral preS1 domain of the L-HBsAg with the cellular
118 receptor sodium taurocholate co-transporting polypeptide (NTCP) [6]. HBsAg is also
119 involved in the assembly and secretion of progeny virions and, most importantly, can
120 be secreted in absence of capsid and the HBV genome, forming spherical or
121 filamentous subviral particles (SVPs) that exceed the number of circulating virions by
122 3-6 orders of magnitude [4]. Such a high level of circulating HBsAg is thought to
123 represent a viral immunotolerance strategy that can exhaust adaptive immune
124 responses [7]. To circumvent immune exhaustion, removing the tolerogenic HBsAg
125 from circulation could help restore T- and B-cell responses and lead to control of HBV
126 infection [8-10]. An ideal therapeutic strategy may combine immune-stimulatory
127 mechanisms with virus neutralization.

128 HBsAg can be hijacked by HDV, a satellite virus of HBV from the *Kolmioviridae*
129 family with a circular, negative-sense single-strand RNA genome. HDV uses HBsAg
130 for its own envelopment and dissemination and, consequently, the two viruses share
131 the same entry pathway into hepatocytes via NTCP. Therefore, HDV infection occurs
132 only as co- or superinfection with HBV and is associated with the most severe form of
133 viral hepatitis [11, 12]. Here, we characterized a potent and broadly neutralizing mAb
134 that neutralized HBV and HDV infections in vitro, efficiently blocked intrahepatic viral

135 spread and reduced HBsAg as well as HBV and HDV viremia in human liver-chimeric
136 mice.

137 **Materials and Methods**

138 **Isolation of anti-HBs monoclonal antibodies (mAbs) from human memory B cells**

139 The use of blood cells from healthy human HBV vaccinated donors was approved by
140 the ethical committee “*Comitato Etico Canton Ticino*” (Switzerland). All participants
141 gave written informed consent. Individuals were selected based on high anti-HBs
142 serum antibody titer, as tested by a standard diagnostic method. Total IgG⁺ memory B
143 cells were immortalized from peripheral blood mononuclear cells via a previously
144 described method [13]. After 2 weeks of culture, the B cell supernatants were analyzed
145 for their capacity to bind to HBsAg from three serotypes (adw, adr, ayw) by ELISA. To
146 isolate human mAbs, mRNA from B cells of wells with HBsAg binding was reverse
147 transcribed via RT-PCR, cloned, and produced recombinantly as IgG1 via transient
148 transfection into mammalian cell lines. MAbs were affinity-purified using HiTrap Protein
149 A columns and sterilized via passage through 0.22 µm filters.

150

151 **For further details regarding the materials and methods used, please refer to**
152 **supplementary information and CTAT table.**

153 **Results**

154 **Isolation and engineering of a potent anti-HBs human mAb from a vaccinated** 155 **donor**

156 To identify broadly neutralizing antibodies against HBV, we isolated IgG⁺ memory B
157 cells from peripheral blood mononuclear cells of HBV vaccinated donors and
158 immortalized them using Epstein-Barr Virus and CpG [13]. The supernatants from B
159 cell cultures were analyzed for binding to HBsAg by ELISA. Selected mAbs were
160 produced as recombinant IgG1 and neutralized HBV more potently than previously
161 characterized mAbs against preS1 (Ma18/7 [14]), against HBsAg (17.1.41, 19.79.5
162 [15]) or polyclonal HBV immune globulin (HBIG) (**Fig. 1A**). To determine binding
163 behavior, we performed a binding competition assay by ELISA. The original B-cell
164 derived mAb HBC34 showed excellent neutralization capacity and did not compete
165 with the other potently neutralizing mAbs, indicating that it recognizes a distinct
166 functional epitope on HBsAg (**Fig. S1A**). Engineering of the HBC34 Fab light chain
167 variable region for improved developability and introducing two sets of mutations in the
168 Fc generated the preclinical lead VIR-3434 (alias HBC34-dev-LS-GAALIE). The LS Fc
169 mutation (M428L N434S) was introduced to extend in vivo half-life via increased
170 binding to neonatal FcR (FcRn) [16-18] and the GAALIE/XX2 Fc (G236A A330L I332E)
171 to augment effector functions [19-21]. Importantly, these mutations in the Fab and Fc
172 of VIR-3434 did not alter HBsAg binding affinity compared to the parental HBC34
173 antibody (**Fig. 1B**).

174

175 **VIR-3434 neutralizes HBV and HDV infection with pan-genotypic activity in vitro**

176 To assess the *in vitro* neutralizing activity of VIR-3434 against HBV, primary human
177 hepatocytes (PHH) were infected with HBV in the presence of VIR-3434, Ma18/7 or
178 HBIG (**Figs. 1C** and **S1B**). All three antibodies led to a concentration-dependent
179 reduction in HBeAg (**Fig. 1C**) and HBsAg (**Fig. S1B**) secretion, indicating the inhibition
180 of HBV infection and replication. VIR-3434 had a >12,000x lower IC₅₀ value based on
181 HBeAg secretion compared to HBIG (10.9 vs. 138,000 ng/ml) and showed an
182 increased potency (>3x) compared to Ma18/7 (34.9 ng/ml), a mAb that binds to the
183 preS1 region of the L-HBsAg (**Fig. 1C**) [14]. In addition, VIR-3434 efficiently neutralized
184 HDV (genotype 1, enveloped with HBsAg of HBV genotype A) in Huh7-NTCP cells with
185 an IC₅₀ value of 1.4 ng/ml, while Ma18/7 and HBIG neutralized with IC₅₀ values of 13.0
186 ng/ml (>9x higher) and 31,800 ng/ml (>22,000x higher), respectively (**Fig. 1D**). We next
187 investigated the binding of VIR-3434 to HBsAg from all ten HBV genotypes (A-J) in
188 transiently transfected Expi293 cells. VIR-3434 demonstrated pan-genotypic binding
189 activity, with only genotypes B and F demonstrating lower binding levels (**Fig. 1E**). In
190 this qualitative assay, this may be due to the lower expression levels of HBsAg that
191 was detected with these genotypes. To evaluate the breadth of neutralization of VIR-
192 3434, we generated HDV enveloped with HBsAg from eight different HBV genotypes
193 (A-H). VIR-3434 potently neutralized virus harboring all tested HBsAg genotypes with
194 similar potency (IC₅₀ range 1.4 – 4.2 ng/mL), including genotype B and F (**Fig. 1F**).
195 Taken together, these data show that VIR-3434 potently and broadly neutralizes HBV
196 and HDV *in vitro* with pan-genotypic activity.

197

198 **VIR-3434 targets a conserved epitope on the antigenic loop (AGL) of HBsAg**

199 To characterize whether VIR-3434 recognizes a linear or conformational epitope, we
200 analyzed the binding of VIR-3434 to HBsAg preparations produced in yeast or in a
201 human hepatoma cell line (PLC/PRF/5) by Western Blot. VIR-3434 recognized HBsAg
202 only under non-reducing conditions, indicating that VIR-3434 binds to a conformational
203 epitope (**Fig. 2A**). To further map the epitope, we utilized the Chemical Linkage of
204 Peptides onto Scaffolds (CLIPS) technology (Pepscan), which identifies
205 conformational and discontinuous epitopes by constraining antigen-derived peptides
206 to adopt looped structures that mimic the structure of the peptide in context of the full
207 protein [22]. Motif 1 (₁₁₄STTSTGPCRTC₁₂₄) in S-HBsAg was required for VIR-3434
208 binding (**Fig. 2B**), while a second motif 2 (₁₄₅GNCTCIIPSSWAF₁₅₈) stabilized binding
209 (**Fig. 2B**). To evaluate conservation of the epitope in circulating HBV strains, 28,331
210 HBV sequences from the HBV database (HBVdb [23]) were aligned to a reference
211 sequence (GenBank ACU26993.1) using pairwise alignments. Remarkably, >99%
212 conservation was observed for 7 out of 11 amino acids in motif 1 and 12 out of 14
213 amino acids in motif 2, with high conservation and/or conservative substitutions for the
214 remaining positions (**Fig. 2C, S2A and S2B**). Of note, 19 known natural HBV variants
215 or vaccine escape mutants carry mutations within the epitope. VIR-3434 demonstrated
216 binding activity to all but one of the evaluated HBsAg variants by flow cytometry (**Fig.**
217 **2D**). However, binding activity to T123N or the vaccine escape variant G145R was
218 decreased, and binding to the T123N/C124R double-mutation was completely
219 abrogated (**Fig. 2D**) [24, 25]. We next generated HDV enveloped with HBsAg harboring
220 the T123N, T123N/C124R or G145R mutations to assess the ability of VIR-3434 to
221 neutralize these mutants. Despite decreased binding to T123N in flow cytometry (**Fig.**

222 **2D**), VIR-3434 efficiently neutralized this mutant (**Fig. 2E**). In contrast, the
223 T123N/C124R double mutant was not neutralized at the antibody concentrations
224 tested but also demonstrated a 6-fold reduced viral titer during production of the virus
225 stock compared to WT or the other HBsAg mutants (**Fig. 2F**). The removal of the
226 cysteine at position 124 may destabilize the antigenic loop and possibly result in
227 reduced viral fitness, as observed previously [26]. Additionally, VIR-3434
228 demonstrated decreased neutralization of the G145R variant. Among the 28,331
229 sequences available in the HBVdb, the C124R and G145R mutations were only
230 detected at low frequencies of 0.03% and 0.77%, respectively. Overall, VIR-3434 binds
231 to a highly conserved conformational epitope in the HBsAg antigenic loop, resulting in
232 broad pan-genotypic neutralizing activity.

233 **Potential mechanism of VIR-3434-mediated HBV neutralization**

234 A hallmark of CHB is the persistence of high HBsAg levels in the blood. The high
235 excess of HBsAg subviral particles (SVPs) may act as a decoy, competing with
236 antibodies binding to infectious virions. We thus assessed the *in vitro* neutralization
237 capacity of VIR-3434 in the presence of exogenous HBsAg SVPs derived from yeast
238 or PLC cells. Western Blot confirmed that the yeast-derived HBsAg contained only S-
239 HBsAg, whereas PLC cell supernatant contained a mixture of S-, M- & L-HBsAg (**Fig.**
240 **S3**). Both forms of exogenous HBsAg competed with VIR-3434 neutralization, resulting
241 in a shift (up to 25-fold) of the neutralization IC₅₀ (**Fig. 3A**). At concentrations
242 exceeding 1 µg/ml, competition could be overcome to achieve complete neutralization,
243 even in the presence of 2,000 IU/ml of HBsAg.

244 Next, we set out to investigate the mechanism of action by which VIR-3434
245 inhibits HBV infection. The attachment of HBV to hepatocytes is mediated by i) low-
246 affinity binding of HBsAg to HSPGs—cell-surface glycoproteins modified by
247 glycosaminoglycans (GAG) interacting with the preS1 domain and the antigenic loop
248 of HBsAg—and ii) by high-affinity binding of preS1 to the entry receptor NTCP [5]. We
249 exposed HepG2-NTCP cells to HBV in the presence or absence of VIR-3434 and
250 quantified cell-bound virus by quantitative PCR. Neither VIR-3434, the entry inhibitor
251 Myrcludex B [27], nor the preS1-targeting mAb Ma18/7 were able to block binding of
252 HBV to NTCP-expressing cells. Only heparin, a soluble, sulfated GAG could efficiently
253 block attachment of HBV to host cells (**Fig. 3B**), as previously reported [5]. These data
254 indicate that VIR-3434 neutralizes HBV infection not via blocking GAG-mediated
255 attachment but at a later step of infection.

256 We next compared the neutralization capacity of the full VIR-3434 IgG molecule
257 with that of its Fab fragment. While VIR-3434 IgG potently neutralized HBV, VIR-3434
258 Fab did not (**Fig. 3C**), suggesting that high-avidity bivalent binding of VIR-3434 IgG to
259 virions may be required to block viral entry. However, surface plasmon resonance
260 (SPR) experiments showed that VIR-3434 IgG and Fab have comparable or only
261 modestly different apparent affinities to immobilized HBsAg ($K_{D,app} < 1$ nM for IgG vs.
262 $< 1-8$ nM for Fab) (**Fig. 3D, Suppl. Table S1**). Therefore, an alternate explanation is
263 that neutralization by VIR-3434 requires the cross-linking of virions to form antibody-
264 antigen immune complexes, which is mediated by full-length IgG but not the Fab
265 fragment. To test this hypothesis, we analyzed the morphology of yeast-derived HBsAg
266 in complex with VIR-3434 by negative stain electron microscopy. The untreated HBsAg
267 control as well as HBsAg incubated with the VIR-3434 Fab contained symmetrical,

268 monodispersed, spherical SVPs of ~20 nm diameter (**Fig. 3E**). In contrast, pre-
269 incubation of HBsAg with VIR-3434 IgG induced cross-linking of SVPs (**Fig. 3E**). In
270 summary, HBV neutralization requires VIR-3434 as a full-length IgG and is likely
271 mediated via sequestration of virions into SVP-containing immune complexes.

272 **HBC34/VIR-3434 prevents HBV spread and decreases circulating HBsAg *in vivo*** 273 **in liver-chimeric mice**

274 Based on its potent and broad *in vitro* neutralization capacity, we tested the
275 effectiveness of HBC34/VIR-3434 in an *in vivo* model of HBV infection. As the specific
276 tropism of HBV for human hepatocytes does not allow infection of wild-type mice, we
277 used a well-established human liver-chimeric mouse model, in which PHH are
278 transplanted into the liver of uPA/SCID beige (USG) mice that lack B and T cells [27,
279 28]. These mice were infected intraperitoneally with HBV (genotype D) and treated with
280 1 mg/kg HBC34 (the parental mAb of VIR-3434 with human Fc) or 1 mg/kg HBIG twice
281 per week or 1 µg/ml oral in drinking water of NRTI Entecavir (ETV) during the viral
282 spreading phase starting at 3 weeks post-infection (**Fig. 4A**). After 9 weeks of infection
283 (6 weeks of treatment), serum levels of HBV DNA (i.e., viremia) had increased ~3 logs
284 above baseline (BL) in mice that were not treated or were treated with HBIG or a control
285 mAb. ETV reduced HBV viremia to below detectable levels at 3 and 6 weeks of
286 treatment, while administration of HBC34 limited HBV viremia to below BL (**Figs. 4B**
287 **and S4A**). Serum HBsAg levels increased ~2 logs above BL during the treatment
288 period in the control and HBIG treated groups (**Figs. 4C and S4B**). HBC34 led to a
289 reduction in serum HBsAg levels to ~1 log below BL at 6 weeks of treatment. Although
290 HBC34 binds to the antigenic loop of HBsAg, concentrations of up to 50 µg/ml of

291 HBC34 did not interfere with the assay used for HBsAg quantification (Abbott Architect
292 System) in this study (**Fig. S4C**). Analysis of livers after 6 weeks of treatment also
293 demonstrated high efficacy of HBC34 to prevent the increase of intrahepatic viral loads
294 (HBV pgRNA, total HBV RNA, and total HBV DNA), as values were comparable to
295 those at the start of treatment (**Fig. 4D**). In contrast, treatment with HBIG at the same
296 dose as HBC34 did not limit the intrahepatic increase of HBV DNA and RNA. HBV core
297 antigen (HBcAg) staining of mouse liver sections from untreated animals showed a
298 dramatic spread of HBV from few infected cells at 3 weeks post-challenge to infection
299 of nearly all liver-resident human hepatocytes after 9 weeks (**Fig. 4E**). While HBIG
300 showed similar levels of HBcAg positive cells compared to untreated controls, HBC34
301 or ETV treatment limited intrahepatic spread of HBV (**Fig. 4E**). Given the relatively low
302 potency of HBIG in comparison to HBC34/VIR-3434 (~1,000-12,000x less potent, see
303 **Figs. 1A and 1C-D**), the lack of efficacy of HBIG in this study was likely due to the sub-
304 effective dose of HBIG administered to the mice [29]. Our results show that
305 HBC34/VIR-3434 is highly efficacious *in vivo* at blocking the infection of new
306 hepatocytes, thereby limiting the increase in viremia and reducing the level of
307 circulating HBsAg.

308 **HBC34/VIR-3434 reduces HBV and HDV viremia in chronically infected human** 309 **liver-chimeric mice**

310 We next tested the ability of HBC34/VIR-3434 to reduce HBV infection in human liver-
311 chimeric mice with an established infection with high HBV viremia and antigenemia.
312 Mice were infected with HBV for 8 to 12 weeks (see **Fig. 5**) and either not treated or
313 treated with HBC34 (1 mg/kg), with the approved polymerase inhibitor Lamivudine

314 (LAM) at 0.4 mg/ml in drinking water, or with a combination of HBC34 and LAM (**Fig.**
315 **5A**). Both drugs alone reduced HBV DNA viremia (~1 log) after 6 weeks of treatment
316 (**Figs. 5B** and **S5A**). The combination of HBC34 and LAM showed enhanced
317 effectiveness in decreasing viremia compared to the individual agents (greater than 2
318 logs after 2 weeks treatment and at all subsequent timepoints). Treatment with HBC34
319 alone achieved a significant reduction of serum HBsAg levels (nearly 2 logs below BL
320 after 6 weeks of treatment); as expected, serum HBsAg levels in LAM treated mice
321 remained close to BL levels throughout the course of treatment (**Figs. 5C** and **S5B**).
322 Notably, the two HBC34 treated animals with lower HBsAg BL levels showed higher
323 reduction and achieved undetectable levels of HBsAg (**Fig. S5B**).

324 We then investigated the efficacy of HBC34 in the context of HBV/HDV co-
325 infection. Human liver-chimeric mice that had been stably co-infected with both HBV
326 and HDV (10 weeks after challenge) were treated with HBC34 (1 mg/kg,
327 intraperitoneally twice per week) either carrying wild-type constant regions of the
328 original human IgG1 or a murine IgG2a, which matches the murine Fc γ Rs expressed
329 in this model (**Fig. 5D**). Importantly, human or murine HBC34 reduced both HBV (2.7
330 log or 1 log reduction) and HDV (2.4 log or 2.6 log reduction) viremia within 2-4 weeks
331 of treatment (**Figs. 5 E-F** and **S5**). In addition, both mAb variants significantly reduced
332 serum HBsAg levels (2.9 log or 2.5 log reduction) (**Figs. 5G** and **S5**). Intrahepatic total
333 HBV DNA, HBV RNA transcripts, and HDV RNA did not change substantially in
334 HBC34-treated mice, indicating that the mAb treatment did not reduce the number of
335 infected cells in this model (**Fig. S5G**). Taken together, our data demonstrate that
336 HBC34/VIR-3434 is highly effective in reducing viremia and circulating HBsAg in
337 mouse models of chronic HBV infection and HBV/HDV co-infection.

338 Discussion

339 In acute viral infections, the use of mAbs is well-established in prophylaxis
340 against respiratory syncytial virus infection of neonates or in post-exposure prophylaxis
341 against Rabies virus infection [30]. For early therapy, potent neutralizing mAbs were
342 recently approved against Ebola virus infections [31], and the Coronavirus Disease
343 2019 (Covid-19) pandemic showcased that mAbs provide clear clinical benefits [32].
344 For therapy targeting chronic viral infections, several ultrapotent, broadly neutralizing
345 mAbs are in clinical development against human immunodeficiency virus 1 [30]. In this
346 study, we set out to discover a broadly neutralizing mAb for treatment of CHB. We
347 identified the human mAb VIR-3434 binding with high affinity to HBsAg and neutralizing
348 HBV with broad potency. VIR-3434 had a >12,000-fold higher potency than HBIGs in
349 vitro, which are well-established as post-exposure prophylaxis for newborns to HBV+
350 mothers [33] as well as in preventing re-infection following liver transplantation [34]. In
351 exploratory clinical studies of patients with CHB, a monthly high dose of HBIG reduced
352 HBsAg levels and induced seroconversion to anti-HBs positive after one year in few
353 individuals with low BL HBsAg (<500 IU/ml) [29, 35]. Monoclonal anti-HBs antibody
354 therapies have been previously evaluated and found to be safe in early, small clinical
355 trials. A human anti-HBs AGL mAb, GC1102, effectively lowered HBsAg by 2 to 3 log₁₀
356 IU/mL and was well tolerated in a Phase 1 study in patients with CHB, with no evidence
357 of serious sequelae, such as immune complex disease [36]. A mixture of two human
358 anti-HBs AGL mAbs, HBV-AB^{X^{TL}} (HepeX-B), significantly reduced HBsAg and HBV-
359 DNA and had a favorable safety and tolerability profile at up to 4 doses of 80 mg
360 administered weekly, with no signs of immune complex disease or hepatotoxicity in 27

361 patients with CHB [37]. Thus, anti-HBs mAbs may provide promising treatment options
362 for patients with CHB.

363 We found that VIR-3434 binds to a conformational epitope within the antigenic
364 loop of HBsAg, which forms dimers that protrude from the surface of SVPs and virions
365 [38]. The epitope consists of two highly conserved non-overlapping regions. VIR-3434
366 binding and neutralization was confirmed for all HBV genotypes (A-H) and a variety of
367 naturally occurring HBsAg variants. In this study, reduced in vitro activity was observed
368 for 2 of 19 HBsAg variants tested (T123N/C124R and G145R). The T123N/C124R
369 double-mutation was associated with a reduced viral fitness as reflected by a reduced
370 HDV titer in vitro, consistent with published results [26]. G145R is a well-described
371 vaccine-escape mutation, which has been observed in infants born to HBV+ mothers
372 who received the HBV vaccine (with or without HBIGs) [39]. The general prevalence
373 of G145R is low ([Suppl. Fig. S2](#)) and correlates with specific genotypes C and G.
374 However, published studies selected G145R upon treatment of HBV-infected liver-
375 chimeric mice with HBsAg-specific mAbs [40]. Of note, we did not observe viral
376 rebound in any of the liver-chimeric mice treated with HBC34/VIR-3434 throughout the
377 course of the study. The emergence of mAb-selected HBV escape mutants in patients
378 with CHB may be mitigated by combination treatment of mAb with additional
379 therapeutic agents (e.g. NRTI) to inhibit ongoing replication during the course of
380 treatment.

381 HBV enters the hepatocyte in a two-step process: initial reversible attachment
382 to HSPGs via the preS1 and AGL regions of HBsAg [5, 41], then the myristoylated
383 preS1 region interacts with the NTCP receptor with high affinity [6]. VIR-3434 neither
384 blocked the interaction of HBsAg with HSPGs nor inhibited viral attachment, indicating

385 no interference with the preS1 region binding to NTCP. Yet the neutralizing activity of
386 VIR-3434 is similar to Ma18/7, currently one of the most potent preS1-specific
387 neutralizing mAbs [42]. Compared to the preS1 epitope of Ma18/7 only present on L-
388 HBsAg of virions and SVP filaments, the epitope of VIR-3434 has higher abundance
389 in vivo, as it is present on the AGL in L-, M- and S-HBsAg on virions, filaments, and
390 spherical SVPs. This comparison of neutralization potency and epitope abundance
391 suggests either a much higher affinity of VIR-3434 or a mode of neutralization that
392 does not require blocking every epitope on HBV virions. We further observed that
393 neutralization by VIR-3434 requires the full-length IgG. In contrast, the Fab
394 demonstrated no neutralizing activity despite binding HBsAg with high affinity. Thus,
395 we hypothesize that the cross-linking of virions and SVPs into immune complexes, as
396 we observed in TEM imaging, is likely required for VIR-3434-mediated neutralization.

397 In addition to neutralizing viral entry, the formation of immune complexes leads
398 to the clustering of mAb Fcs that is required for high avidity interaction with low-affinity
399 FcγRs. Fc-mediated effector functions may explain the HBC34/VIR-3434-mediated
400 rapid reduction of HBsAg (up to 2 logs) and HBV viremia (1 log) within 2-6 weeks of
401 treatment of chronically HBV-infected human liver-chimeric USG mice. VIR-3434 may
402 opsonize HBsAg SVPs and infectious virions and target them to FcγR-expressing
403 phagocytes, such as monocytes, macrophages, dendritic cells or neutrophils. In a prior
404 study, an anti-HBs mAb (CRL-8017) accelerated the uptake of HBsAg into primary
405 human monocytes, classical dendritic cells, neutrophils, and B cells in vitro [43].
406 Similarly, treatment with an anti-HBs murine mAb (E6F6) led to rapid clearance of
407 serum HBsAg (>2 log) and HBV DNA via Kupffer cells, macrophages, and neutrophils
408 in several mouse models of HBV infection [44]. As interactions with FcγRs mediated

409 the clearance of HBsAg and virions [44], effector functions are likely pivotal for mAb-
410 mediated therapy of CHB.

411 To extend serum half-life, the Fc region of VIR-3434 was engineered to include
412 the LS mutation to increase IgG1 binding to human FcRn in endosomes and thus
413 increase recycling into the serum [16-18]. In addition, the Fc region of VIR-3434 was
414 engineered using the GAALIE/XX2 mutation to increase binding to human activating
415 FcγRIIa and IIIa, while decreasing binding to the inhibitory FcγRIIb [19]. This
416 engineered Fc is designed to boost phagocytosis of HBsAg and HBV virions and
417 augment antigen presentation. Previously, the GAALIE/XX2-modified Fc of an anti-HA
418 stem antibody increased the prophylactic potency against influenza A virus infection
419 compared to the mAb carrying a human wild-type Fc in mice transgenic for human
420 FcγRs and FcRn [20]. This increased protection was mediated via fast priming of naïve
421 CD8⁺ T cells, providing evidence for a mAb-mediated “vaccinal effect”. In human
422 transgenic FcγR and FcRn mice, the GAALIE Fc increased protection in therapeutic
423 models of mAbs against SARS-CoV-2 virus infection [21] or cancer metastasis in the
424 lung [19]. While the tested wild-type human and mouse Fcs of HBC34/VIR-3434 both
425 can interact with the murine FcγRs and FcRn, the GAALIE mutation specifically
426 increases binding only to human FcγRs. The USG mouse model lacking T and B cells
427 and carrying myeloid effector cells with mouse FcγRs thus is not suited to address the
428 benefits of the GAALIE Fc. Future studies using primary human immune cells in vitro,
429 mouse models transgenic for human FcγRs and FcRn, and clinical trials will need to
430 determine whether VIR-3434 can induce a vaccinal effect and mediate long-term anti-
431 HBV immunity.

432 Beyond HBV mono-infection, more than 12 million chronic HBV carriers are co-
433 infected with HDV worldwide and have an even higher risk of developing liver cirrhosis
434 and hepatocellular carcinoma. Current treatment options are limited to PEG-IFN α ,
435 which is associated with only a low frequency of sustained responses, and daily
436 injection of the viral entry inhibitor bulevirtide that has gained conditional marketing
437 approval in the EU [11]. Here, we show that VIR-3434 neutralizes HDV infection in vitro
438 and efficiently reduces HDV viremia in human liver-chimeric USG mice. Entry inhibition
439 and removal of HDV virions/HBsAg from circulation support the potential of VIR-3434
440 to be an efficacious treatment for patients chronically co-infected with HBV and HDV.

441 In conclusion, VIR-3434 is a highly potent, half-life extended and effector
442 function-enhanced mAb that binds the antigenic loop present in all forms of HBsAg and
443 neutralizes HBV as well as HDV across all genotypes. VIR-3434 integrates three
444 potential modes of action: (a) inhibition of HBV and HDV entry into new hepatocytes,
445 (b) reduction of circulating HBsAg, and (c) delivering HBsAg to antigen-presenting cells
446 that could reinvigorate adaptive T and B cell responses and long-term immunity via a
447 vaccinal effect. Consequently, the half-life extended VIR-3434 provides a promising
448 therapeutic option for the treatment of patients chronically infected with HBV or also
449 co-infected with HDV and has the potential to induce a functional cure.

450

451

452 **Abbreviations**

Anti-HBs	Antibody directed against HBsAg
AGL	Antigenic loop
BL	Base line
BLI	Bio-layer interferometry
CHB	Chronic hepatitis B
ETV	Entecavir
FcRn	Neonatal Fc receptor
FcγRs	Fc gamma receptors
HBIG	Hepatitis B immunoglobulins
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HDV	Hepatitis delta virus
HSPG	Heparan sulfate proteoglycan
mAb	Monoclonal antibody
NRTIs	Nucleos(t)ide reverse transcriptase inhibitors
NTCP	Sodium taurocholate co-transporting polypeptide
PEG-IFNα	Pegylated-interferon alpha
PHH	Primary human hepatocytes
SD	Standard deviation
SVP	Subviral particle
TEM	Transmission electron microscopy
USG	uPA/SCID beige

453

454

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460

461 **Author contributions**

462 Conceived study: F.A.L., E.C., F.B., M.D., D.C., M.A.S. Isolated and characterized
463 mAbs: E.C., F.B., F.Z., S.B., G.L., S.J., Performed in vitro virological experiments:
464 F.A.L., J.Z., H.K., H.I., A.S. Performed in vivo experiments and virological
465 measurements: T.V., M.L. SPR/EM experiments: L.E.R., D.M.B. Bioinformatic
466 analysis: L.B.S., A.T. Manuscript writing: F.A.L, T.V., F.B., J.N., M.D., D.C., M.A.S.
467 Supervision and manuscript editing: N.P., S.U., A.T., A.L.C., G.S., L.A.P., C.M.H.,
468 M.D., D.C. and M.A.S.

469 **Competing interests**

470 F.A.L, E.C., F.B., J.Z, L.E.R., J.N., F.Z., H.K., S.B., G.L., S.J., H.I., L.B.S., N.P., M.L.,
471 A.T., A.L.C., G.S., L.A.P., C.M.H., D.C., M.A.S are employees of Vir Biotechnology
472 and may hold shares in Vir Biotechnology. L.A.P. is a former employee and
473 shareholder in Regeneron Pharmaceuticals. D.M.B. received funding from Vir
474 Biotechnology. The remaining authors declare that the research was conducted in the
475 absence of any commercial or financial relationships that could be construed as a
476 potential conflict of interest. SU is inventor and holder on patents protecting bulevirtide.

477 **Data availability statement**

478 All source data that support the findings of this study are available from the
479 corresponding authors upon reasonable request.

480

481

482 **Figure Legends**

483 **FIGURE 1. Pan-genotypic neutralization of HBV and HDV infection.** (A) HBV
484 neutralization of a panel of human mAbs using HepaRG cells. *Hepatitis B Immune
485 Globulin (HBIG) was tested at 1000x higher concentrations 5,000, 500, and 50
486 µg/ml. (B) ELISA binding of HBC34 development variants to HBsAg serotype adw. (C)
487 Neutralization of HBV (genotype D) measured via HBeAg secretion as marker of
488 infection of primary human hepatocytes (PHH) in the presence of VIR-3434, Ma18/7
489 mAb or HBIG. (D) Immunostaining for HDAg of Huh7-NTCP cells infected with HDV
490 (genotype 1, enveloped with HBsAg of HBV genotype A) in the presence of respective
491 antibodies. (E) Flow cytometry binding of VIR-3434 to Expi293 cells transfected with
492 HBsAg of genotypes A-J. (F) VIR-3434 neutralizing HDV enveloped with HBsAg of 8
493 different HBV genotypes on Huh7-NTCP cells.

494 **FIGURE 2. VIR-3434 binds a conserved epitope of the HBsAg antigenic loop.**
495 (A) Western Blot with HBsAg separated under non-reducing or reducing conditions
496 and probed with HBC34 for detection. (B) Epitope mapping using a library of linear
497 and looped peptides (CLIPS technology). The VIR-3434 epitope consists of two
498 motives (blue) in the antigenic loop of HBsAg. (C) Epitope conservation plots based
499 on HBV sequence data from HBVdb. Residues of virus variants colored based on VIR-
500 3434 neutralization (green) or loss of neutralization (red); grey variants were not
501 tested. Only frequencies of >2% were visualized. (D) Binding via flow cytometry of
502 VIR-3434 to HBsAg variants transiently expressed in Expi293 cells. (E) Neutralization
503 of HDV enveloped with the respective HBsAg variants. (F) Infectious titer by titration
504 of HDV enveloped with wildtype HBsAg (genotype D) or one of three virus variants on

505 HuH7-NTCP cells. Shown geometric mean \pm SD of two independent experiments;
506 statistically significant differences relative to WT by one-way ANOVA (p -value* ≤ 0.05).

507 **FIGURE 3. VIR-3434 IgG neutralizes infection and aggregates HBsAg in immune**
508 **complexes.** (A) HBV virus stock (50 IU HBsAg/mL) premixed with indicated
509 concentrations of HBsAg was incubated with VIR-3434 and added to primary human
510 hepatocytes for infection. (B) Binding of HBV to HepG2-NTCP cells in the presence of
511 inhibitors quantified by qPCR. Shown is the mean \pm SD of nine data points from a
512 single experiment. Statistical differences were analyzed by one-way ANOVA. p -value
513 **** $p \leq 0.0001$. (C) HBV neutralization using VIR-3434 IgG or Fab on primary human
514 hepatocytes. (D) SPR analysis of VIR-3434 IgG or Fab binding to immobilized HBsAg.
515 (E) Yeast-derived HBsAg (1,500 IU/mL) was pre-incubated with 5 μ g/mL VIR-3434
516 IgG or the equivalent molar amount of isolated VIR-3434 Fab fragments and imaged
517 by negative-stain electron microscopy.

518 **FIGURE 4. HBC34/VIR-3434 prevents HBV spread and decreases circulating**
519 **HBsAg in liver-chimeric mice.** (A) Human liver-chimeric USG beige mice were
520 infected with HBV (genotype D). Three weeks post infection, at the onset of viral
521 spreading phase, treatment was started twice weekly at 1 mg/kg intraperitoneally with
522 (i) HBC34 (the parental mAb of VIR-3434), (ii) HBIG or (iii) a control mAb or were
523 treated with Entecavir (ETV) at 1 μ g/ml in drinking water. Treatment was continued
524 until week 9 post HBV inoculation, when viral infection was spread throughout the
525 human hepatocytes. (B) HBV viremia (HBV DNA) and (C) HBsAg were assessed in
526 serum by qPCR and ELISA, respectively. The mice were sacrificed 9 weeks post
527 infection and intrahepatic HBV pgRNA, total HBV RNA (HBx region), and total HBV
528 DNA (D) were assessed by (RT-)qPCR. Liver sections were immunostained (E) for

529 HBcAg and CK18 as marker for human hepatocytes. In (D), each circle represents
530 one animal. Shown is the median \pm range. Statistical differences relative to the
531 untreated control were analyzed by one-way ANOVA. p-value * $p \leq 0.05$, ** $p \leq 0.01$.

532 **FIGURE 5. HBC34/VIR-3434 reduces HBV and HDV viremia as well as circulating**
533 **HBsAg in vivo in chronically infected liver-chimeric mice.** (A) Human liver-
534 chimeric USG mice were infected with HBV (genotype D) for 8-12 weeks until stable
535 infection levels were achieved. Mice were treated for 6 weeks twice per week with 1
536 mg/kg HBC34 (the parental molecule of VIR-3434) intraperitoneally, 0.4 mg/ml
537 lamivudine in drinking water or with both drugs in combination. (B) HBV viremia and
538 (C) HBsAg were assessed in serum by qPCR and ELISA. Graph shows results from
539 2 independent experiments. (D) Human liver-chimeric mice were co-infected with HBV
540 (genotype D) and HDV (genotype 1) for 10 weeks until stable co-infection was
541 achieved. Mice were treated for 4 weeks with HBC34 either carrying the native human
542 or an engineered murine Fc portion. (E/F) HBV/HDV viremia and (G) HBsAg were
543 assessed in serum by qPCR and ELISA. One animal in the murine Fc group was
544 sacrificed at week 2. Each circle represents one animal. Shown is the mean \pm SD.
545 Statistical differences were analyzed by one-way ANOVA. p-value * $p \leq 0.05$, ** $p \leq$
546 0.01 , *** $p \leq 0.001$, **** $p \leq 0.0001$, ns $p > 0.05$.

547

548 **Supplemental Material Online**

549 **Detailed Methods** can be found online.

550 **Supplemental Figures S1-S5** can be found online.

551 **Supplemental Table S1** can be found online.

552

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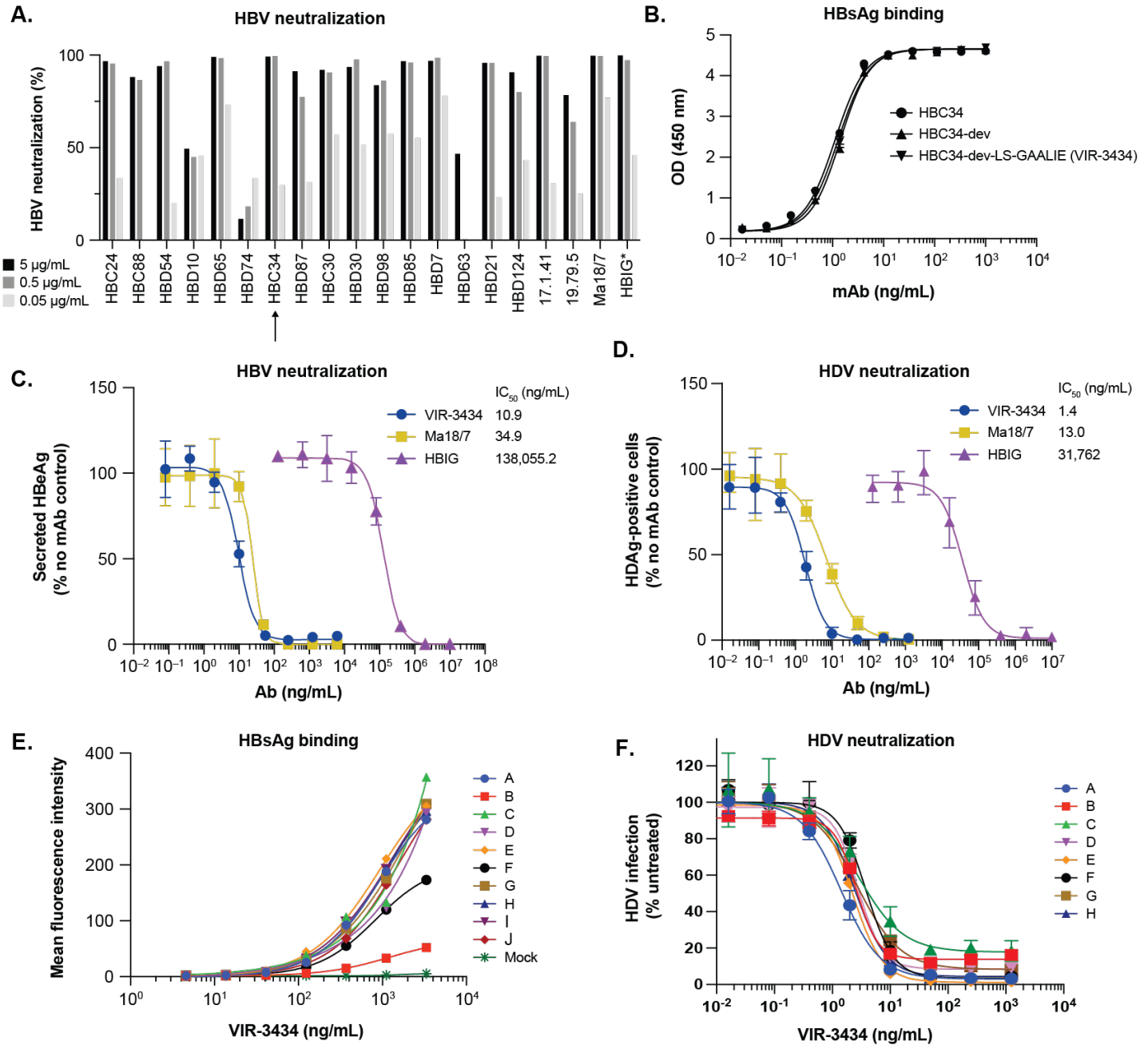


FIGURE 1. Pan-genotypic neutralization of HBV and HDV infection. (A) HBV neutralization of a panel of human mAbs using HepaRG cells. *Hepatitis B Immune Globulin (HBIG) was tested at 1000x higher concentrations 5,000, 500, and 50 µg/ml. (B) ELISA binding of HBC34 development variants to HBsAg serotype adw. (C) Secreted HBeAg as marker of infection of primary human hepatocytes (PHH) with HBV (genotype D) in the presence of VIR-3434, Ma18/7 mAb or HBIG. (D) Immunostaining for HDAg of Huh7-NTCP cells infected with HDV (genotype 1, enveloped with HBsAg of HBV genotype A) in the presence of respective antibodies. (E) Flow cytometry binding of VIR-3434 to Expi293 cells transfected with HBsAg of genotypes A-J. (F) VIR-3434 neutralizing HDV enveloped with HBsAg of 8 different HBV genotypes on Huh7-NTCP cells.

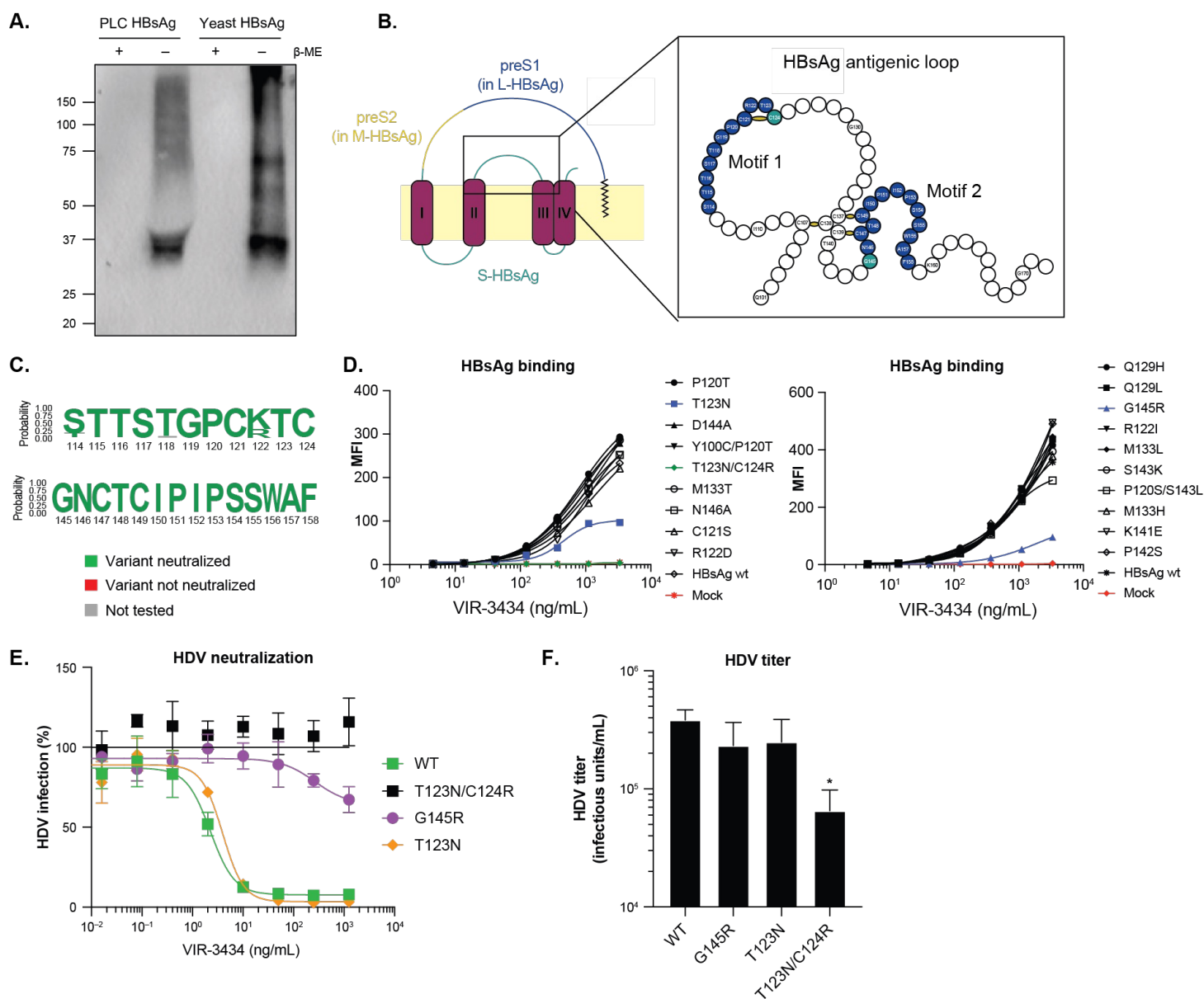


FIGURE 2. VIR-3434 binds a conserved epitope of the HBsAg antigenic loop. (A) Western Blot with HBsAg separated under non-reducing or reducing conditions and probed with HBC34 for detection. (B) Epitope mapping using a library of linear and looped peptides (CLIPS technology). The VIR-3434 epitope consists of two motives (blue) in the antigenic loop of HBsAg. (C) Epitope conservation plots based on HBV sequence data from HBVdb. Residues of virus variants colored based on VIR-3434 neutralization (green) or loss of neutralization (red); grey variants were not tested. Only frequencies of >2% were visualized. (D) Binding via flow cytometry of VIR-3434 to HBsAg variants transiently expressed in Expi293 cells. (E) Neutralization of HDV enveloped with the respective HBsAg variants. (F) Infectious titer by titration of HDV enveloped with wildtype HBsAg (genotype D) or one of three virus variants on HuH7-NTCP cells. Shown geometric mean \pm SD of two independent experiments; statistically significant differences relative to WT by one-way ANOVA (p -value* \leq 0.05).

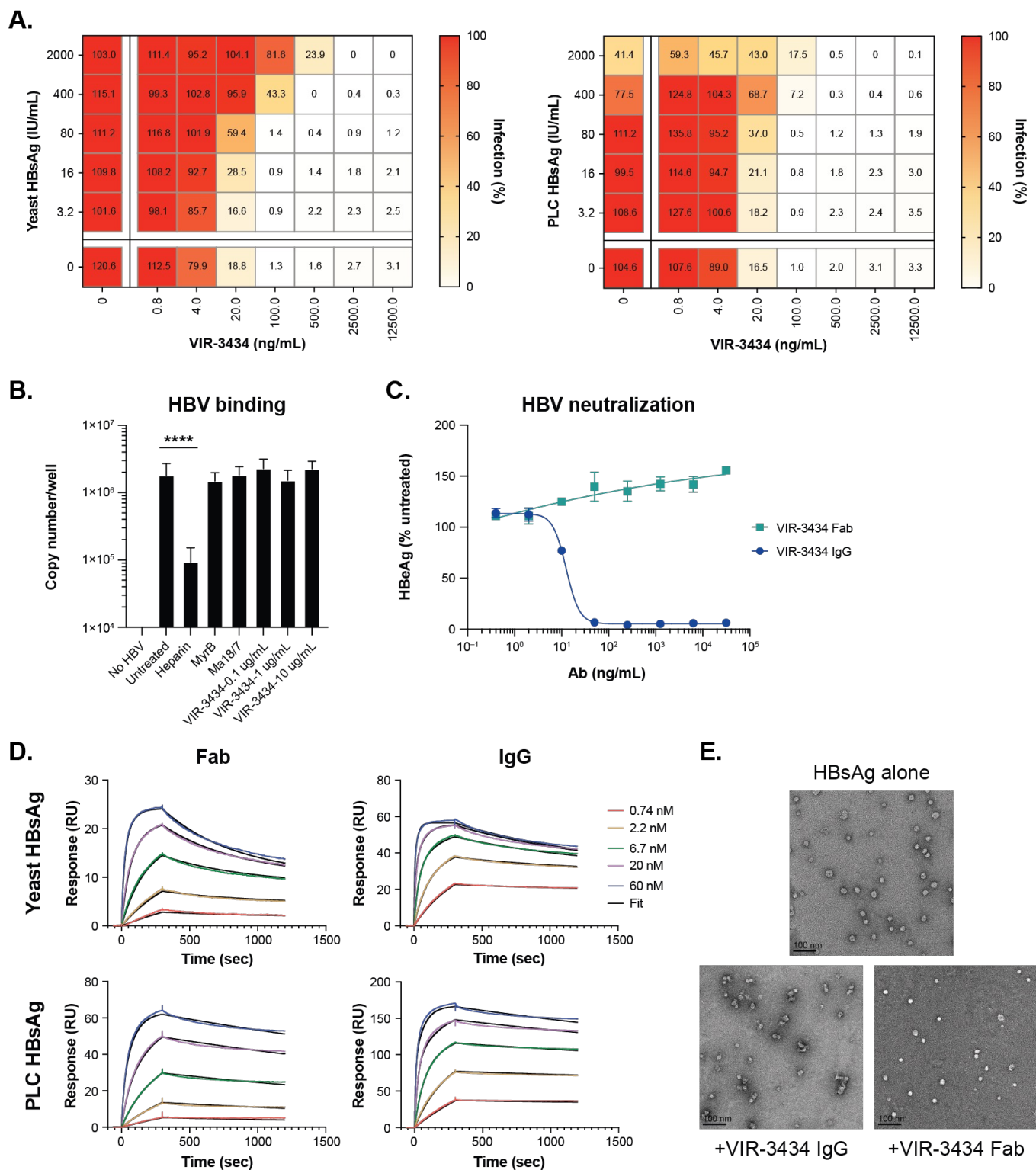


FIGURE 3. VIR-3434 IgG neutralizes infection and aggregates HBsAg in immune complexes. (A) HBV virus stock (50 IU HBsAg/mL) premixed with indicated concentrations of HBsAg was incubated with VIR-3434 and added to primary human hepatocytes for infection. (B) Binding of HBV to HepG2-NTCP cells in the presence of inhibitors quantified by qPCR. Shown is the mean \pm SD of nine data points from a single experiment. Statistical differences were analyzed by one-way ANOVA. p -value **** $p \leq 0.0001$. (C) HBV neutralization using VIR-3434 IgG or Fab on primary human hepatocytes. (D) SPR analysis of VIR-3434 IgG or Fab binding to immobilized HBsAg. (E) Yeast-derived HBsAg (1,500 IU/mL) was pre-incubated with 5 μ g/mL VIR-3434 IgG or the equivalent molar amount of isolated VIR-3434 Fab fragments and imaged by negative-stain electron microscopy.

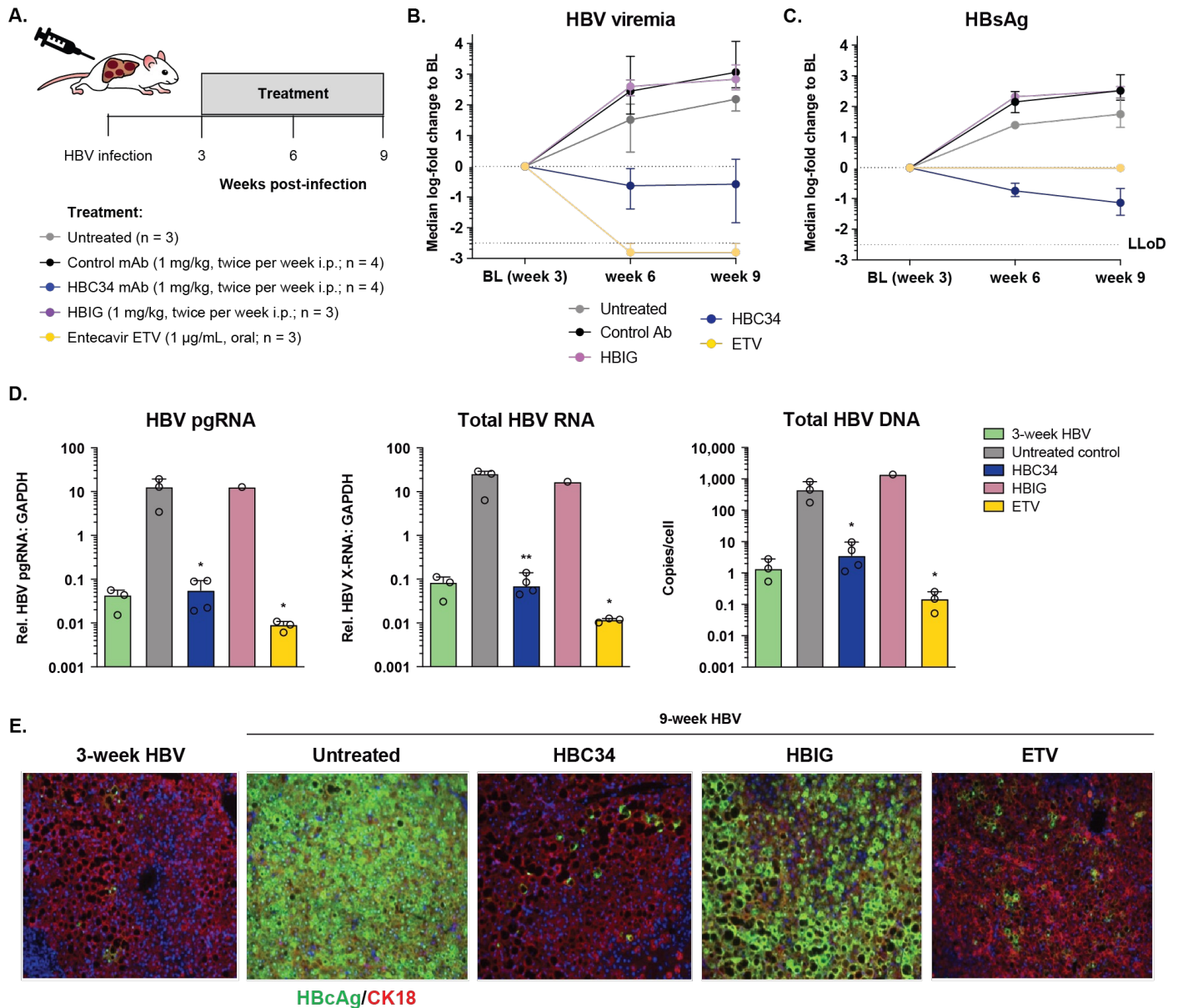


FIGURE 4. HBC34/VIR-3434 prevents HBV spread and decreases circulating HBsAg in liver-chimeric mice. (A) Human liver-chimeric USG beige mice were infected with HBV (genotype D). Three weeks post infection, at the onset of viral spreading phase, treatment was started twice weekly at 1 mg/kg intraperitoneally with (i) HBC34 (the parental mAb of VIR-3434), (ii) HBIG or (iii) a control mAb or were treated with Entecavir (ETV) at 1 µg/ml in drinking water. Treatment was continued until week 9 post HBV inoculation, when viral infection was spread throughout the human hepatocytes. (B) HBV viremia (HBV DNA) and (C) HBsAg were assessed in serum by qPCR and ELISA, respectively. The mice were sacrificed 9 weeks post infection and intrahepatic HBV pgRNA, total HBV RNA (HBx region), and total HBV DNA (D) were assessed by (RT-)qPCR. Liver sections were immunostained (E) for HBcAg and CK18 as marker for human hepatocytes. In (D), each circle represents one animal. Shown is the median ± range. Statistical differences relative to the untreated control were analyzed by one-way ANOVA. p-value * $p \leq 0.05$, ** $p \leq 0.01$.

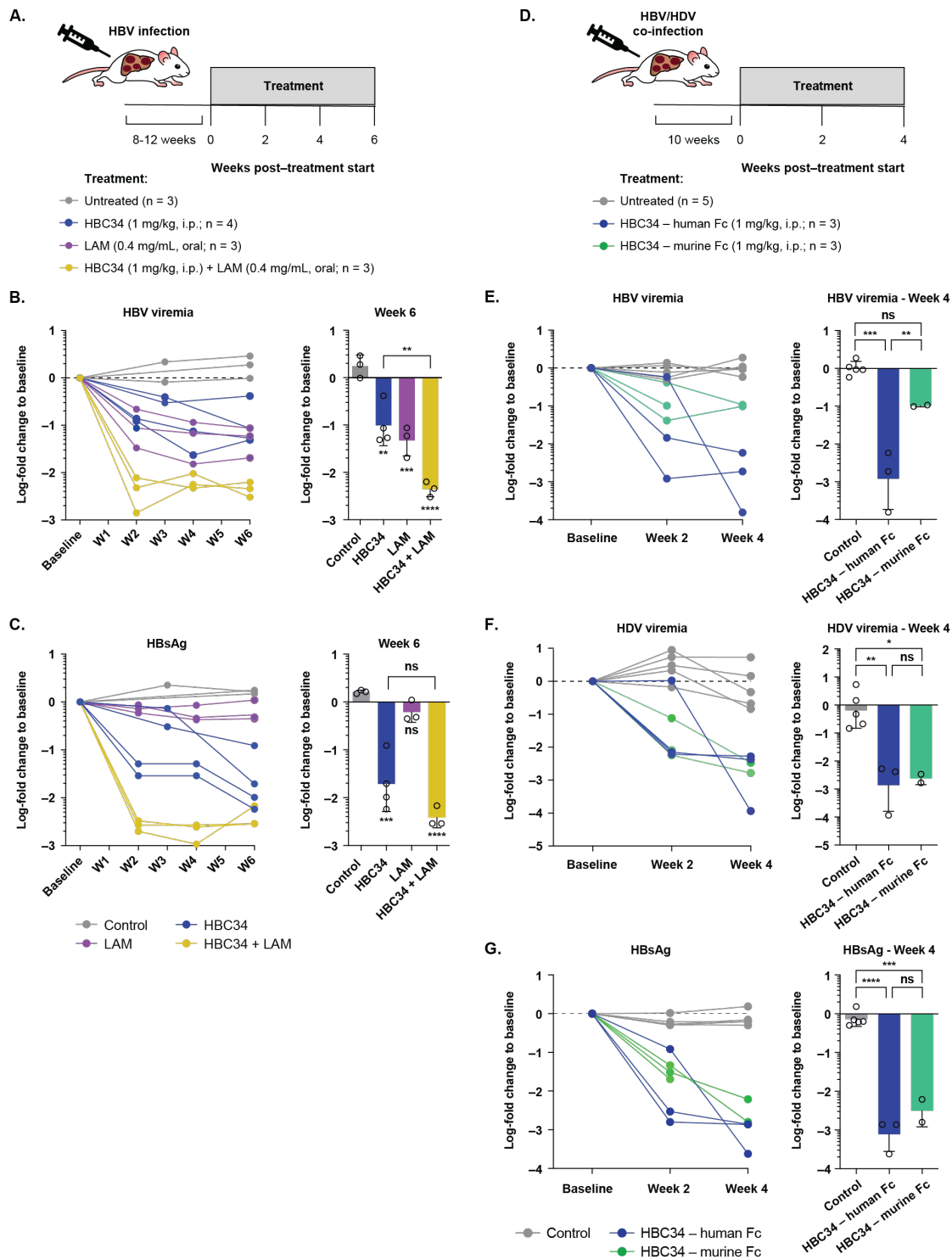


FIGURE 5. HBC34/VIR-3434 reduces HBV and HDV viremia as well as circulating HBsAg in vivo in chronically infected liver-chimeric mice. (A) Human liver-chimeric USG mice were infected with HBV (genotype D) for 8-12 weeks until stable infection levels were achieved. Mice were treated for 6 weeks twice per week with 1 mg/kg HBC34 (the parental molecule of VIR-3434) intraperitoneally, 0.4 mg/ml lamivudine in drinking water or with both drugs in combination. (B) HBV viremia and (C) HBsAg were assessed in serum by qPCR and ELISA. Graph shows results from 2 independent experiments. (D) Human liver-chimeric mice were co-infected with HBV (genotype D) and HDV (genotype 1) for 10 weeks until stable co-infection was achieved. Mice were treated for 4 weeks with HBC34 either carrying the native human or an engineered murine Fc portion. (E/F) HBV/HDV viremia and (G) HBsAg were assessed in serum by qPCR and ELISA. One animal in the murine Fc group was sacrificed at week 2. Each circle represents one animal. Shown is the mean \pm SD. Statistical differences were analyzed by one-way ANOVA. p-value * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, ns $p > 0.05$.