Effect of Peg-IFN on the viral kinetics of patients with HDV infection treated with bulevirtide

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Background & Aims: Bulevirtide is a first-in-class entry inhibitor antiviral treatment for chronic hepatitis D. The viral kinetics during bulevirtide therapy and the effect of combining bulevirtide with pegylated-interferon (Peg-IFN) are unknown.
Methods: We used mathematical modelling to analyze the viral kinetics in two French observational cohorts of 183 patients receiving bulevirtide with or without Peg-IFN for 48 weeks.
Results: The efficacy of bulevirtide in blocking cell infection was estimated to 90.3%, whereas Peg-IFN blocked viral production with an efficacy of 92.4%, albeit with large inter-individual variabilities. The addition of Peg-IFN to bulevirtide was associated with a more rapid virological decline, with a rate of virological response (>2 log of decline or undetectability) at week 48 of 86.9% (95% prediction interval [PI] = [79.7–95.0]), compared with 56.1% (95% PI = [46.4–66.7]) with bulevirtide only. The model was also used to predict the probability to achieve a cure of viral infection, with a rate of 8.8% (95% PI = [3.5–13.2]) with bulevirtide compared with 18.8% (95% PI = [11.6–29.0]) with bulevirtide + Peg-IFN. Mathematical modelling suggests that after 144 weeks of treatment, the rates of viral cure could be 42.1% (95% PI = [33.3–52.6]) with bulevirtide and 66.7% (95% PI = [56.5–76.8]) with bulevirtide + Peg-IFN.
Conclusions: In this analysis of real-world data, Peg-IFN strongly enhanced the kinetics of viral decline in patients treated with bulevirtide. Randomized clinical trials are warranted to assess the virological and clinical benefit of this combination, and to identify predictors of poor response to treatment.
Impact and implications: Bulevirtide has been approved for chronic HDV infection by regulatory agencies in Europe based on its good safety profile and rapid virological response after treatment initiation, but the optimal duration of treatment and the chance to achieve a sustained virological response remain unknown. The results presented in this study have a high impact for clinicians and investigators as they provide important knowledge on the long-term virological benefits of a combination of Peg-IFN and bulevirtide in patients with CHD. Clinical trials are now warranted to confirm those predictions.

Introduction

Chronic HBV infection is a major global burden affecting approximately 300 million people worldwide.[1] Although antiviral treatments are effective to suppress viral replication and reduce the risk of liver disease, they do not lead to viral eradication, and infected cells continue to produce viral proteins such as HBsAg.[2]

A major complication of HBV infection is the coinfection at the acute stage or superinfection at the chronic carrier stage with HDV. Chronic HDV infection affects at least 15–20 million HBV-carriers worldwide.[3] HDV is a small defective single-stranded RNA virus that requires the presence of empty HBV envelopes carrying HBsAg to infect hepatocytes.[4] Chronic HBV/HDV infection is responsible for the most severe form of chronic viral hepatitis, leading to high rates of cirrhosis, decompensation of cirrhosis, and hepatocellular carcinoma.[5]

Chronic HDV infection (CHD) is still considered as an orphan disease in most parts of the world and the therapeutic options to treat its related liver disease are limited. Until recently the only, yet unapproved, treatment was pegylated interferon (Peg-IFN),[6,7] which has shown poor tolerance and disappointing efficacy, with high rates of relapse after treatment cessation,[8,9] even after several years of treatment.[8] The heterogeneity of patients response to Peg-IFN makes it difficult to estimate its precise impact on HDV RNA kinetics, with estimates of viral undetectability after 48 weeks of treatment ranging from 13% to 41%,[8,10–14] and a rate of virological response (defined as a decline superior to 2 log from baseline or undetectable HDV RNA at week 48) of approximately 40%.[12] In 2020, bulevirtide (BLV, previously known as Myrcludex B) has been conditionnally approved by the European Medicines Agency at a dose of 2 mg/day subcutaneously (Hepcludex®). BLV is a synthetic lipopeptide corresponding to the 47 N-terminal amino acids of the pre-S1 domain of the large HBsAg protein. It is a first-in-class entry inhibitor that acts by blocking the binding of HDV envelope to the sodium/bile acid cotransporter (NTCP), a membrane receptor responsible for the entry of both HBV and HDV into the hepatocytes through its fixation with the HBV envelope.[15] In phase III clinical trials, BLV at a dose of 2 mg daily has shown promising results, with a rate of virological response of 71% and a rate of viral undetectability of 12% after 48 weeks of treatment.[16] Only few results have been published regarding viral dynamics after treatment cessation, but they suggest that the vast majority of patients experience relapse.[17,18] Studies are ongoing to evaluate virological and clinical effects on longer periods.

BLV has been approved by regulatory agencies as a monotherapy, and the combination of BLV and Peg-IFN has not yet been clinically evaluated in large studies. The virological response of the combination in randomized clinical trials have been so far heterogeneous with rates of viral undetectability ranging from 44% in MYR204[19] to 60% in MYR203[20] after 48 weeks of treatments, but both of these results were obtained on a small number of patients.[21,22] In France, where off-label treatments can be reimbursed by the healthcare system, BLV can be used either alone or in combination with Peg-IFN, at the prescriber's discretion. Patients receiving BLV have been enrolled in two separate, multicentre, retrospective, and prospective cohorts, from which we obtained a unique dataset, allowing us to compare the viral kinetics in patients receiving BLV alone or in combination with Peg-IFN.

Mathematical models have been largely used to decipher and predict the outcome of treatments against hepatitis B, C, or D.[23–33] In the case of HCV, these tools provided important insights regarding treatment duration and the time needed to cure viral infection, i.e. achieve a sustained virological response.[25,34,35] Such models can be used to emulate various therapeutic strategies. In this modelling analysis, we aimed to characterize the viral kinetics in patients treated for 48 weeks with BLV alone or in combination with Peg-IFN. We predicted the

potential of the combination to improve the rate of viral cure with longer term administration.[23,25,34,36]

Materials and methods

Design of the study
We used data from two French multicentric cohort studies that assessed the efficacy of BLV alone or in combination with Peg-IFN alfa: (1) the French Early Access Program (cATU) and (2) the ANRS HD EP01 BuleDelta study (NCT04166266). The following analysis was performed gathering the data from those two cohorts.

The cATU study was a prospective cohort study, conducted between September 2019 (initiation of the compassionate access program of BLV in France) and September 2020 (conditional marketing authorization) and funded by MYR GmbH (now Gilead Sciences). It included patients with CHD with detectable HDV RNA or anti-HDV antibodies. Inclusion criteria were adults with either a compensated cirrhosis or a high fibrosis (>F3 evaluated with liver biopsy or Fibroscan®), or moderate fibrosis (F2) with persistent cytolysis (alanine transaminase [ALT] values > 2 × upper limit of normal for >6 months). BuleDelta study has been initiated in 2019 and was funded and sponsored by ANRS|MIE. BuleDelta study started the inclusions in February 2020 and is still ongoing with prospective and retrospective inclusions of patients with CHD already treated or with an indication for treatment with BLV. Inclusion criteria were adults with compensated liver disease with detectable HDV RNA in plasma or serum.

In both cases, BLV was administered at a dose of 2 mg subcutaneously once daily, as a monotherapy (BLV group) or in combination with Peg-IFN (BLV + Peg-IFN group) once weekly subcutaneously at a dose of 45–180 μg weekly according to physician's choice. Treatment changes, such as the cessation of BLV or Peg-IFN, as well as dose adjustments could be made at the physician's discretion throughout the study. As inclusions could be retrospective in both cases, some patients were enrolled in both studies (Fig. 1). All patients either gave written informed consent or received an information note to participate in these studies.

Data included in the viral kinetic analysis
Our viral kinetic analysis focused on the virological and biochemical responses during the first 48 weeks of treatment. HDV RNA, HBV DNA, ALT, and HBsAg were measured at week 0 (BLV initiation), 4, 8, 12, 24, 36 and 48 (Fig. S1). Baseline clinical characteristics included age, sex, BMI, HBeAg-status, the presence or absence of cirrhosis, concomitant medication with nucleos(t)ide analogs (NUCs), Fibroscan® and platelet count (Table 1, Figs. S2 and S3).

Data obtained after 48 weeks of treatment were excluded, and all data provided before 48 weeks (on- and off-treatment) were taken into account in our analysis (Fig. S4). Patients that were already receiving anti-HDV treatment (e.g. Peg-IFN) at the time of inclusion were excluded. We focused our analysis on patients receiving BLV 2 mg (alone or in combination) and data obtained after a potential dose adjustment to 10 mg were excluded. Doses of Peg-IFN were not available for all patients and therefore were not taken into account in the main analysis. However, a subanalysis was performed using only patients for whom the dose was available (Table S1, Figs. S5 and S6). Viral measurements and quantification levels were heterogeneous, with lower limit of quantification (LLOQ) ranging from 100 to
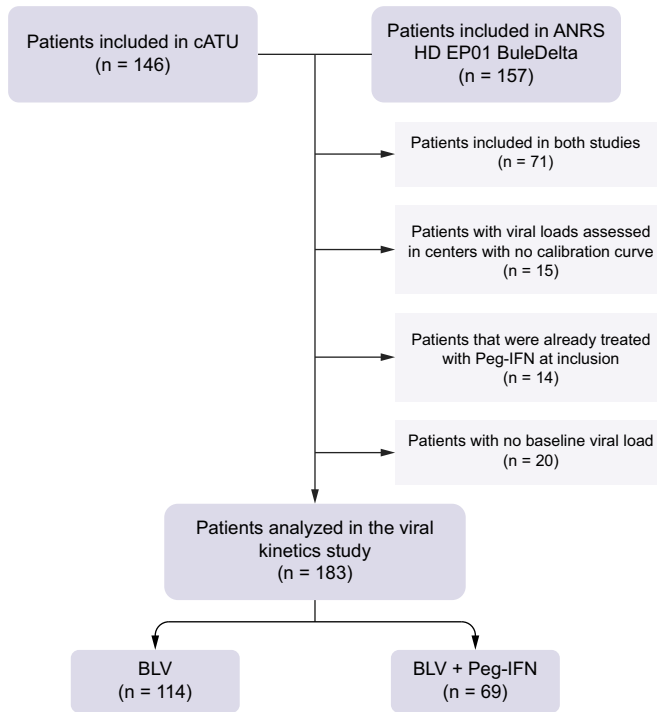
Fig. 1. Flowchart of the viral kinetics study. BLV, bulevirtide; Peg-IFN, pegylated interferon.

1,000 IU/ml depending on the centre in which the data were analyzed (Table S2).

Calibration curves provided by the French National Reference Center on HDV (Figs S7 and S8) were used to ensure comparability in HDV RNA measurements across centres. Data originating from centres for which no calibration curves were available were not analyzed.

Model of HDV and ALT dynamics during BLV therapy

The model is a standard target cell limited model with a pool of target cells (T, HBV mono infected cells) that can be infected by HDV virions (V) at a rate β and become HDV-HBV coinfected cells (I). We assumed that T was constant over the study period infection, remaining at its pre-treatment steady-state value, T_0. BLV acts by blocking cell entry with effectiveness noted η, infected cells are lost at rate δ, and produce p virions per day, which are cleared at rate c (Eqs 1 and 2):

$$\frac{dI}{dt} = ß(1-\eta)\, VT_0 - \delta I \tag{1}$$

$$\frac{dV}{dt} = pI - cV \tag{2}$$

$$\frac{dA}{dt} = s + a\delta I - c_a\, A \tag{3}$$

Following the model developed by Ribeiro et al.,[37] we also incorporated the dynamics of ALT (A). In this model, ALT is produced at a zero order (s) by dying cells, and are therefore proportional (a) to the loss rate of both HDV/HBV infected cells (δ) and susceptible cells (Eq. 3). Assuming that the number of cell infections was small after treatment initiation, A could be expressed using an analytical solution, where c_a, A_0 and A_∞ are the clearance, the value at baseline and the value in absence of infection of ALT, respectively (Eq. 4).

$$A(t) = A_\infty \left[1 - \frac{A_0 - A_\infty}{A_\infty\,(c_a - \delta)} \left(\delta e^{-c_a t} - e^{-\delta t} \right) \right] \tag{4}$$

To ensure model identifiability, a number of hypotheses were done. First, as HDV infection is generally associated with suppression of HBV replication, we neglected the impact of HBV viral replication on HDV kinetics. We also assumed that all hepatocytes were infected with HBV[38] and the total population of HDV susceptible cells was therefore equal to $T_0 = c\,\delta/\beta p$.[39] Without loss of generality[40,41] the infectivity rate (β) and the rate of production of HDV RNA by infected cells (p) were also fixed to 10^{-7} ml.RNA^{-1}·day^1 and 10 RNA cell^{-1}·day^{-1}.[40] Considering that no early HDV RNA observations were available after treatment initiation, the clearance of HDV RNA was also fixed to 0.24 day^{-1} based on previous analysis.[41] At baseline, the concentration of virions (V_0) was estimated on a logarithmic scale.

Table 1. Baseline characteristics of the patients included in the analysis.

	Missing data (N)	BLV (n=114)	BLV+Peg-IFN (n=69)	*p* value[*]
Clinical characteristics				
Age[†], years		42 [35–51]	42 [36–51]	0.8
Female[‡]		37 (32)	23 (33)	0.9
BMI[†], kg/m^2	26	25.1 [22.3–27.6]	24.5 [22.5–28.2]	0.8
HIV[‡]	2	21 (19)	8 (12)	0.2
Liver stiffness measurement (FibroScan®)[†], kPa	64	12 [9–19]	11 [8–17]	0.3
Cirrhosis[‡]		83 (73)	52 (75)	0.7
Platelets[†], per cm^3	5	144 [83–196]	163 [122–230]	0.01
Co-medication with NUC[‡]		97 (85)	51 (74)	0.06
Viral and biochemical characteristics				
HBeAg-negative[‡]	35	74 (85)	56 (92)	0.2
HDV RNA[†] (log$_{10}$ IU/ml)		6.5 [5.3–6.9]	6.7 [5.5–7.3]	0.3
Undetectable HBV DNA[‡]	15	88 (81)	36 (60)	0.03
HBsAg[†] (log$_{10}$ IU/ml)	111	3.7 [2.9–4.1]	3.7 [3.4–4.1]	0.5
ALT[†] (IU/L)	3	78 [51.8–119]	84 [54.5–143]	0.5
Follow up[†], days		335 [273–358]	341 [331–331]	0.2

ALT, alanine transaminase; BLV, bulevirtide; NUC, nucleos(t)ide analogs; Peg-IFN, pegylated interferon.
[*] Wilcoxon test; Pearson's test.
[†] Median [IQR].
[‡] n (%).

Estimating effects of Peg-IFN

No *a priori* hypothesis was made for Peg-IFN, which could either block viral production, enhance the loss rate of target cells and/or infected cells, or block cell infection with a pharmacological delay fixed to 8.5 days.[41] For the latter, a multiplicative effect was assumed for the addition of Peg-IFN to BLV. The efficacy of Peg-IFN and/or BLV were set to 0 after treatment interruption. To include the drug effects, a forward selection was performed, where at each step, the model providing the best improvement of likelihood was kept (Table S3), and the procedure continued until no significant improvement was found, using a likelihood-ratio test (LRT, p < 5%).

After the selection of the drug effects, only those that were properly estimated (relative standard error <50%) were kept.

Building the model of inter-patient variability

After inclusion of the drug effects, a screening was performed to test for association between baseline characteristics and individual parameters. Only baseline covariates with <15% missing data were tested. Patients for whom covariates information were missing were excluded in this step. Continuous covariates were transformed into categorical covariates, based on the median observed in the population. Significant association (p <1% on a Wilcoxon test) were retained. On those pre-selected associations, a forward procedure based on a LRT (p <5%) was then done, followed by a backward selection (p <1%) to include the most significant covariate effects.

Treatment discontinuation

We estimated the probability for each treatment to be discontinued, using an exponential survival model (Fig. S9). A joint model was also tested to evaluate the association between the virological response and the probability of treatment discontinuation.

Statistical model

HDV RNA log-transformed observations and ALT levels were simultaneously fitted from the solution of our model. The longitudinal data were analyzed with a population approach using non-linear mixed effect models. The model used to describe for each individual i (i = 1, ..., N) the jth observation (j = 1, ..., N_i), in the kth marker at time t_{ijk} was:

$$Y_{ijk} = f(\theta_i, t_{ijk}) + e_{ijk}$$

with θ_i the vector of parameters of individual i and e_{ijk} the residual error of patient i at observation j for each k marker, assumed to follow a normal distribution centred on zero and a variance equal to σ^2. We assumed an additive error on the HDV log-transformed observations, and tested based on an LRT both a combined and proportional error on ALT levels.

Fixed effects were described by μ the vector of population parameters, whereas η_i was the vector of random effects describing inter-individual variability, with variance-covariance matrix Ω^2, and z_i the vector of individual baseline covariates.

All disease parameters followed a log-normal distribution, and the treatment effects followed a logit-normal distribution. Parameter estimation was performed using a stochastic approximation expectation–minimization (SAEM) algorithm implemented in Monolix software, version 2018 R2 (www.lixoft.com).

Predicting virological responses with long-term BLV administration

We focused our analysis on the virological response, defined as a virological decline of more than 2 logs or undetectable HDV RNA (with a limit of quantification of 2 $\log_{10}$ IU/mL) and the combined response, defined as a virological and a biochemical response (normalization of transaminases with ALT values lower than 40 IU/L).

Using the parameters estimated, we calculated the proportion of patients achieving a response after 48, 96, and 144 weeks of therapy. We compared the outcome in a group treated with monotherapy, with the combination, or with the combination for 48 weeks and the monotherapy afterwards (designated as intermediate strategy).

Results were given assuming either that (i) all patients would stay on treatment (per protocol scenario) or (ii) they could discontinue therapy (intention-to-treat scenario) with the same rate than observed in our data (see above). In the latter, treatment efficacy was set to 0 after treatment interruption.

We also used the model to project the cure of viral infection. For this purpose, we used the concept of cure boundary developed in HCV,[34,36] which is the theoretical viral concentration corresponding to having less than one virus particle in the whole body ($<10^{-4.22}$ IU/ml).

To account for variability, 500 simulations were done using the same number of patients and the patients' characteristics as in the original dataset in each group, and we reported the median, 5 and 95 percentiles. The simulation was performed using Simulx software (www.lixoft.com).

Results

Patients included and population characteristics

A total of 183 patients were included in our viral kinetic analysis, 114 treated with BLV alone and 69 with BLV + Peg-IFN (Fig. 1). Most patients were males (68% and 67% in the monotherapy and combination therapy group, respectively) with a median age of 42 years and negative for HBeAg (85% and 92% in the monotherapy and combination therapy group, respectively). The majority of patients had cirrhosis (73% and 75% in the monotherapy and combination therapy group, respectively), whereas coinfection with HIV was present in 19% and 12% in the monotherapy and combination therapy groups, respectively. Overall, and to the exception of the platelet counts, which was significantly higher in the combination group (p = 0.01), the baseline clinical characteristics were balanced between the two groups (Table 1). At inclusion, the proportion of patients that were chronically treated with NUCs was slightly higher in the monotherapy group than in the combination treatment group (85% and 74%, respectively, p = 0.06). Consequently, the proportion of undetectable HBV DNA was significantly higher (p = 0.03) in the group treated with monotherapy (81% and 60% in the BLV and BLV + Peg-IFN groups, respectively). There were no significant differences in viral levels in both groups, with median levels of HDV RNA, ALT, and HBsAg in the monotherapy group of 6.5 log IU/mL, 78 U/L, and 3.7 log IU/mL, respectively, compared with 6.7 log IU/mL, 84 U/L, and 3.7 log IU/mL in the combination treatment group, respectively.

Data included in the viral kinetic analysis

Among the 183 patients included at baseline, 150 received a treatment for the total duration of the study (48 weeks). The

majority (N = 131) continued treatment with no interruption, and 19 had one or more temporary interruptions. A total of 33 patients terminated treatment before week 48 (Fig. 2).

Among the 80 patients that received BLV + Peg-IFN throughout the whole analysis, the exact dose of Peg-IFN received was informed at all times in 46 patients. Among these patients, 1, 10, 6 and 29 patients received a dose of 45, 90, 135 and 180 mg/kg at treatment initiation, respectively. The dose was modified at least one time in 10 patients and the detail is given in Figs. S5 and S6.

Virological and biochemical response during therapy
In both groups, the virological (HDV RNA) and biochemical (ALT) changes from baseline were statistically significant after 48 weeks of treatment (p <0.0001), whereas no significant change was observed in the levels of HBsAg and HBV DNA (Figs. S10 and S11). The virological response (defined as a decline in viral load >2 log or undetectability) was significantly more frequent in patients receiving BLV + Peg-IFN, with rates equal to 29.3% after 4 weeks, 83.9% after 12 weeks, 85.2% after 24 weeks, 79.2% after 36 weeks, and 83.3% after 48 weeks, compared with 7.8%, 32.2%, 52.6%, 54.7%, and 66.2% (all p <0.01) in patients receiving BLV alone, respectively.

Accordingly, the proportion of undetectable HDV RNA was higher in the combination group, with rates equal to 10.3%, 47.5%, 64.9%, 62.3%, and 68.5% after 8, 12, 24, 36, and 48 weeks respectively, compared with 5.6%, 18.4%, 26.8%, 34.9, and 36.5% in
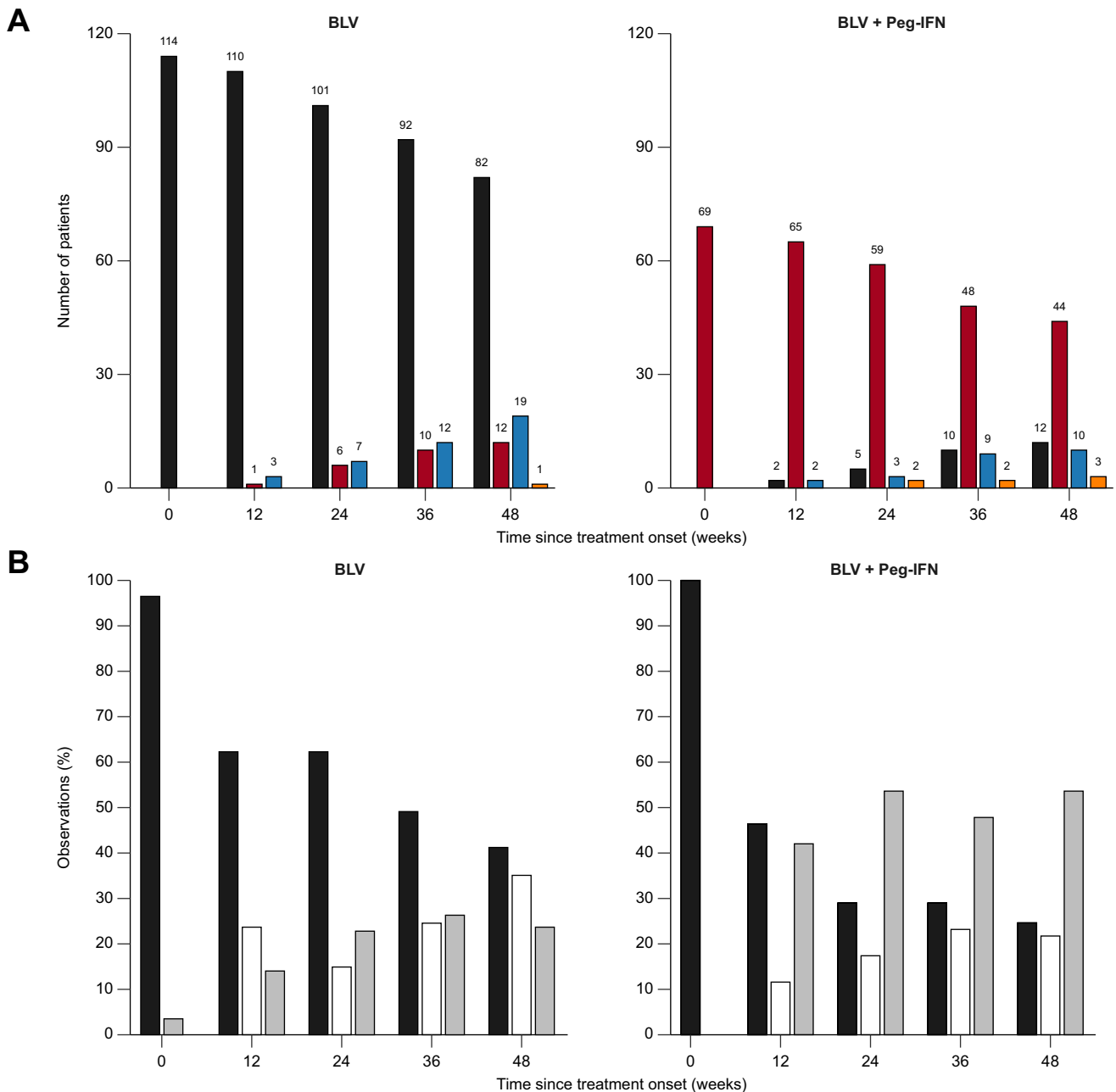
Fig. 2. Patient treatments and observations. (A) Number of patients treated with BLV alone (black), BLV + Peg-IFN (red), Peg-IFN alone (orange) and off-treatment (blue). (B) Corresponding proportion of observations. Black bars correspond to detectable HDV RNA, gray bars represent undetectable HDV RNA, white bars represent the proportion of unavailable data at each time. BLV, bulevirtide; Peg-IFN, pegylated interferon.

the monotherapy group. No significant difference was observed in the level of transaminases between the two groups. The observed virological declines are shown in Fig. 3.

Viral kinetics parameters and antiviral effect of BLV and Peg-IFN

The efficacy of BLV in blocking cell infection was estimated to 90.3%, albeit with a large uncertainty (95% CI = [28.3–99.5]). The main mechanism of action of Peg-IFN was blocking viral production, with an efficacy estimated to 92.4% (95% CI = [73.4–98.2]). It is noteworthy that this estimated value corresponds to a mean estimate in the population, which masks large variability across individuals. Indeed, the efficacy of BLV and Peg-IFN was <50% in 29% and 21% of individuals, respectively, suggesting that these treatments had very limited antiviral activity in a substantial proportion of patients (Figs. S12 and S13). No additional effect of Peg-IFN on the loss rates of susceptible or infected cells, nor on blocking cell infection was found (Table S3).

Consequently, the model predicts that BLV monotherapy leads to a monophasic viral decline (Fig. 4), driven by the loss rate of infected cells (δ), estimated to 0.030 day^{-1}, corresponding to a half-life of 23 days (Table 2). In contrast, patients treated with BLV + Peg-IFN mostly had a biphasic decline, with a first phase driven by the clearance of free virions (c), fixed to be 0.24 day^{-1}, corresponding to a half-life of 2.9 days, and a final phase driven by δ.

With less infected cells over time, ALT was cleared at a rate (c_a) equal to 1.56 day^{-1}, corresponding to a half-life of 0.44 day, and declined continuously from a level of 102 (A_0) to 42 IU/L (A_∞) in young males (defined as younger than 42 years of age). We estimated a 0.76-fold decrease and a 1.3-fold increase in the levels of ALT at baseline, respectively, in females and older patients (p <0.01). The dynamics of ALT was not impacted by the drugs and no differences across groups could be highlighted (Figs. S10 and S11).

Prediction of virological response after 48–144 weeks of treatment

The addition of Peg-IFN to BLV translated into an improved response in the long-term.

In the per protocol scenario, assuming no treatment cessation, the rate of virological response after 48 weeks was predicted to be 56.1% (95% prediction interval [PI] = [47.4–66.7]) in patients treated with BLV, and 86.9% (95% PI = [79.7–95.0]) in patients treated with BLV + Peg-IFN. These rates would increase progressively to reach after 144 weeks rates of 75.4% (95% PI = [66.7–82.4]) and 95.6% (95% PI = [91.3–100]), respectively
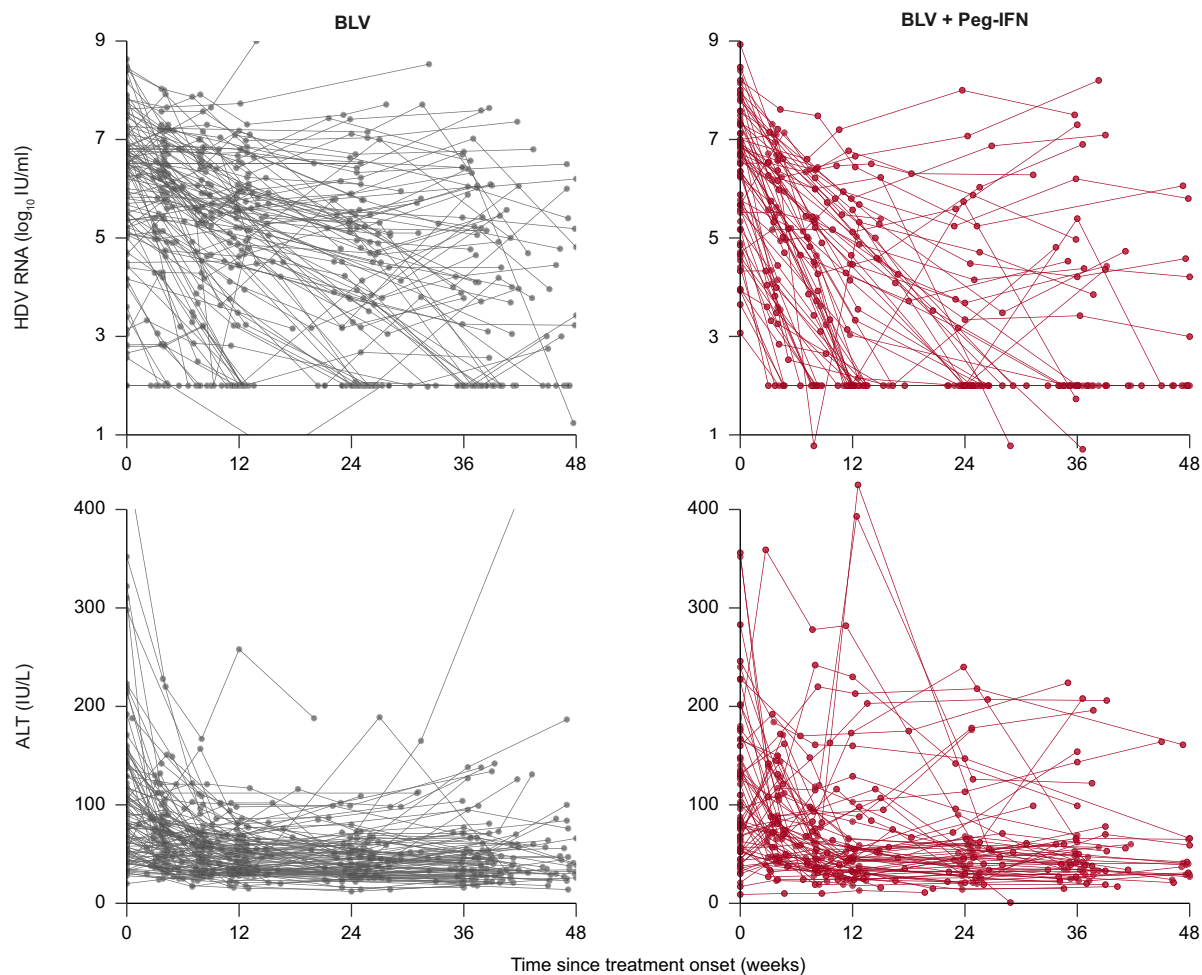

Fig. 3. Viral and biochemical kinetics observed during 48 weeks of treatment with BLV alone (BLV) or in combination with Peg-IFN (BLV + Peg-IFN). ALT, alanine transaminase; BLV, bulevirtide; Peg-IFN, pegylated interferon.
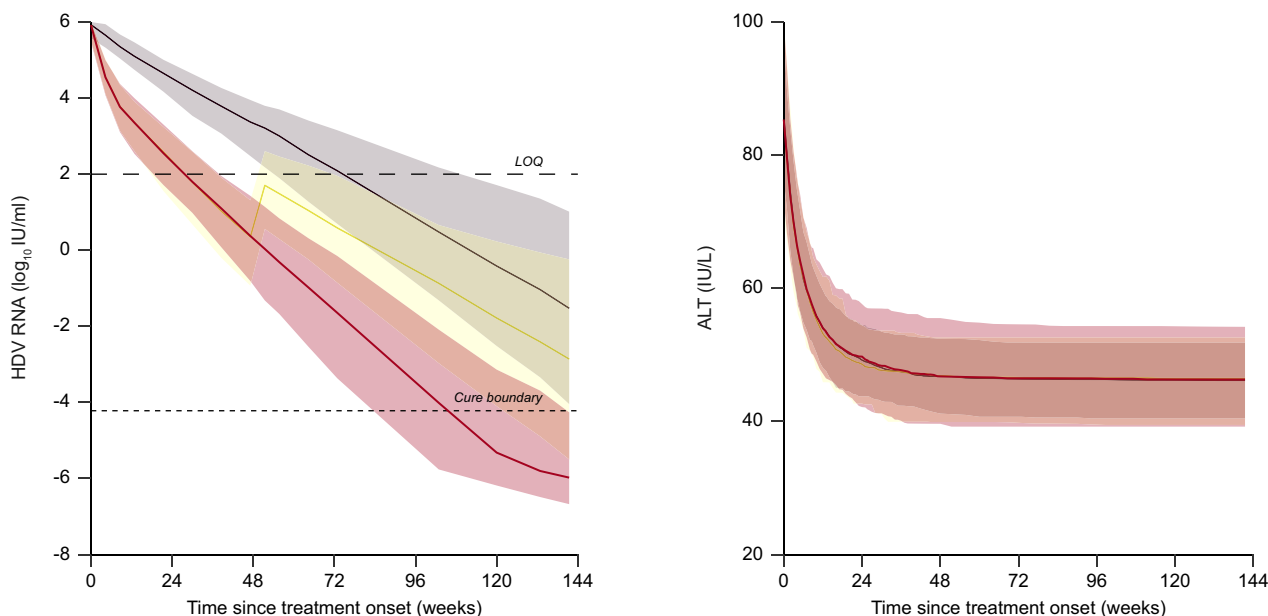
Fig. 4. Long-term viral and biochemical kinetics predicted by the mathematical model in patients receiving BLV (black), BLV + Peg-IFN for 144 weeks (red), and BLV + Peg-IFN for 48 weeks, followed by BLV alone afterwards (yellow). The plain line and its shaded area represent the median prediction of 500 simulated data sets and its 95% prediction interval. ALT, alanine transaminase; BLV, bulevirtide; Peg-IFN, pegylated interferon.

(Fig. 5A). In an intermediate treatment strategy of BLV + Peg-IFN for 48 weeks followed by BLV monotherapy afterwards, the predicted response rate was slightly lower and was equal to 89.8% (95% PI = [82.6–97.1]).

The rates of undetectable HDV RNA were predicted to be 36.0% (95% PI = [26.3–44.7]) and 66.7% (95% PI = [56.5–78.3]) after 48 weeks with BLV monotherapy and BLV + Peg-IFN, respectively. After 144 weeks, the predicted rates were equal to 67.5% (95% PI = [57.9–75.0]), 91.3% (95% PI = [84.0–97.1]) and 78.3% (95% PI = [69.6–88.4]), with BLV monotherapy, BLV + Peg-IFN and the intermediate treatment strategy, respectively.

Similarly, the rates of combined response (defined as a virological and biochemical response) after 48 weeks were predicted to be 21.9 % (95% PI = [14.9–28.9]) and 34.8% (95% PI = [23.2–46.4]) in patients treated with BLV and BLV + Peg-IFN, respectively. After 144 weeks, these rates increased to 29.8%

(95% PI = 21.9–38.6]), 37.7% (95% PI = [26.1–49.3]), and 36.2% (95% PI = [26.1–47.8]) for the monotherapy, the combination and the intermediate treatment strategy, respectively.

In the intention-to-treat scenario, where the risk of treatment discontinuation of both BLV and Peg-IFN is accounted for, these rates were slightly lower. With rates of treatment discontinuation of λ_{BLV} = 0.00075 day^{-1} (95% CI = [0.000570–0.000998]) and λ_{PEG} = 0.0015 day^{-1}, we expect approximately 77% and 60% of patients to be still receiving treatment at 48 weeks with BLV and Peg-IFN, respectively. Consequently, the rates of virological response are expected to decrease accordingly. The details of the virological responses using this scenario it can be found in Fig. 5B and in the Supplementary material.

Of note, regardless of the simulation scenario all treatment strategies led to a similar rate of biochemical response after 48 weeks of treatment (defined as a value of ALT <40 IU/L), with

Table 2. Parameters estimated and their uncertainty, expressed as RSE%.

		Parameter estimate (RSE%)	SD of the random effect (RSE%)
Disease parameters			
δ	Loss rate of infected cells (day^{-1})	3.04 10^{-2} (11.2)	0.718 (12.8)
V_0	Number of virions at baseline (log IU/ml)	5.93 (1.90)	1.39 (6.24)
c_a	ALT clearance (day^{-1})	1.56 (11.6)	0.288 (29.3)
A_0	ALT value at baseline (U/L)	102 (8.11)	0.725 (8.9)
$A_{\infty-young}$	ALT value in absence of infection in young males (IU/L)	43.4 (7.17)	0.542 (6.0)
$A_{\infty-elderly}$	ALT value in absence of infection in elderly males (IU/L)	58.2 (6.64)	
$A_{\infty-Females}$	ALT value in absence of infection in young females (IU/L)	33.1 (8.74)	
Drug effects			
$\varepsilon_{\beta}^{BLV}$	Effect of BLV on blocking infection	0.903 (15.7)	5.48 (43.1)
ε_{p}^{PEG}	Effect of Peg-IFN on blocking viral production	0.924 (5.75)	4.46 (23.2)
Survival analysis			
λ_{BLV}	Rate of BLV discontinuation (day^{-1})	0.00075 (14.4)	
λ_{PEG}	Rate of Peg-IFN discontinuation (day^{-1})	0.00152 (3.39)	
Residual error model			
$a_{HDV\ RNA}$	Additive residual error on HDV RNA (log$_{10}$ IU/ml)	0.807 (3.28)	
b_{ALT}	Proportional residual error on ALT	0.304 (2.79)	

ALT, alanine aminotransferase; BLV, bulevirtide; Peg-IFN, pegylated interferon; RSE%, relative standard error %.
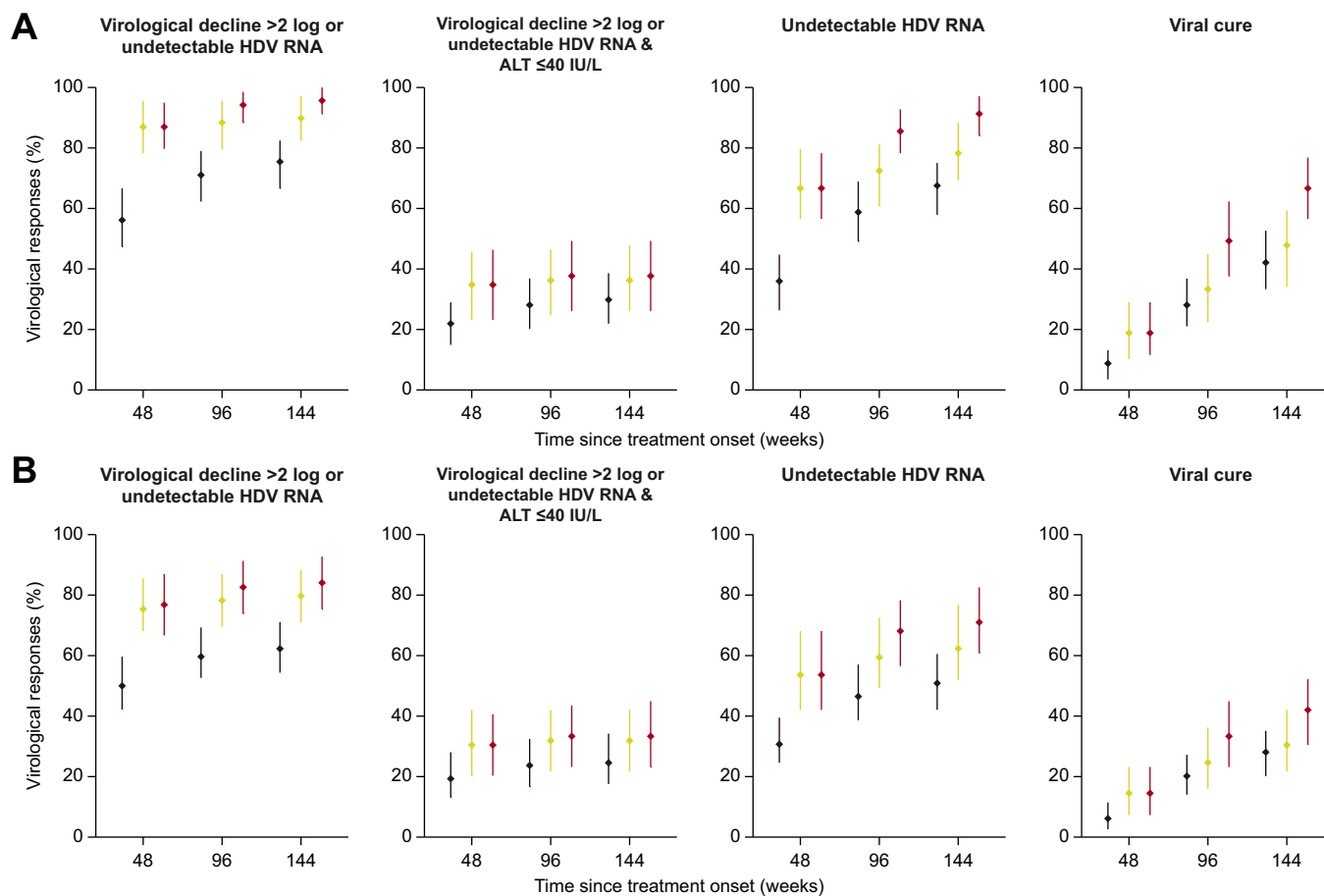
Fig. 5. Viral and biochemical response rate predicted by the mathematical model in patients receiving BLV (black), BLV + Peg-IFN (red), and BLV + Peg-IFN for 48 weeks, followed by BLV alone (yellow), without taking into account treatment discontinuation (per-protocol analysis) (A) and when taken account treatment discontinuation (intention-to-treat analysis) (B). The diamond and its associated line represent the median prediction of 500 simulated data sets and its 95% prediction interval. ALT, alanine transaminase; BLV, bulevirtide; Peg-IFN, pegylated interferon.

rates of 38.6% (95% PI = [29.8–46.5]) and 39.1% (95% PI = [27.5–50.7]) in patients treated with BLV or BLV + Peg-IFN for 48 weeks.

Predicting the proportion of patients achieving HDV cure using the 'cure boundary' concept

Finally, we used the concept of cure boundary developed in HCV[34] to predict the rate of cure of HDV (see Materials and methods). The rate of viral cure after 48 weeks of treatment with BLV was equal to 8.8% (95% PI = [3.5–13.2]), compared with 18.8% (95% PI = [11.6–29.0]) after treatment with BLV + Peg-IFN. These rates would remain low after 96 weeks, with values of 28.1% (95% PI = [21.0–36.8]) and 49.3% (95% PI = [37.7–62.3]), with the monotherapy and the combination, respectively (Fig. 5A). The values obtained with the intermediate treatment strategy would be slightly lower to those obtained with BLV + Peg-IFN, with rates of 33.3% (95% PI = [22.4–44.9]) after 96 weeks of treatment. After 144 weeks of treatment, the predicted rates were equal to 42.1% (95% PI = [33.3–52.6]), 66.7% (95% PI = [56.5–76.8]), and 47.8% (95% PI = [34.0–59.4]), with BLV monotherapy, BLV + Peg-IFN and the intermediate treatment strategy, respectively.

The results of the intention-to-treat scenario are summarized in Fig. 5B and in the Supplementary material.

Discussion

BLV has shown promising results in clinical trials, but little is known about its long-term antiviral activity and the potential benefit of its use in combination with Peg-IFN. The current study provides the first detailed analysis of viral kinetics in patients receiving BLV therapy, alone or in combination with Peg-IFN. Relying on the largest collection of 'real world' data so far, we identified a benefit of Peg-IFN on the virological response that could translate into an improved cure of HDV infection. BLV had a strong antiviral efficacy on blocking cell infection (90.3%), leading to a rate of virological response (defined as a decline of viral load from baseline >2 log or undetectable HDV RNA) of 56% (95% PI = [47–67]) after 48 weeks. Peg-IFN had a complementary mode of action and blocked viral production with an efficacy of 92.4%, leading to a rate of virological response in combination with BLV of 87% (95% PI = [80–95]).

To evaluate our predictions, we compared our results with the virological response after 96 weeks of therapy (N = 54). We found that 55% (CI = [39.3–71.8]) and 72.2% (CI = [51.5–92.9]) in monotherapy and in the combination group, respectively, achieved a virological response at 96 weeks. This is close to the values predicted by our model (59.6 and 82.6%, respectively, see Fig. 5B) and shows that our model provides reliable predictions

of the virological response. In theory, HDV infection, which is caused by an RNA virus without any integrated or episomal form, is curable. We therefore compared our predictions with the rates of sustained virological response (SVR) observed in the MYR204 study,[19] using SVR as a proxy for cure. Our model predicted that 25% of patients could achieve cure after 48 weeks of BLV + Peg-IFN followed by 48 weeks of BLV alone (Fig. 5B) which is close to the SVR rate of 32% (CI = [20–45]) reported in MYR204 (Table S4).

After 144 weeks of treatment, and when accounting for treatment discontinuation, the rates of virological response could reach 80% (PI = [71–88]) and 84% (PI = [75–93]) with the intermediate strategy (BLV + PEG for 1 year followed by BLV alone) and the combination, respectively. This shows that although the predicted rates of virological response is higher in patients treated with BLV + Peg-IFN than in those treated with BLV alone, the benefit of maintaining Peg-IFN could be lower in the long-term. However, a prolonged combined administration of BLV and Peg-IFN over 48 weeks of treatment may provide some additional benefits that were not accounted for by our model, such as the possibility to clear HBsAg.

From a modelling perspective, our model has some limitations. First, it neglects the dynamics of cell turnover and further assumes that all susceptible cells are infected with HBV at baseline. Second, we could not identify an effect of Peg-IFN on the clearance of infected cells, as found in some HBV studies.[27] Whether this is a genuine effect or simply reflects a lack of power to identify it, will need to be studied. Consequently, the dynamics of ALT was predicted to be similar between the two treatment groups as a reflection of our data (Figs. S10 and S11), whereas a slightly higher biochemical response was observed in the combination group in MYR204 study. Further, HBsAg data were missing in many individuals, and precluded its integration in the model.[40] Such data will be important to analyze in the future, and could help identify different populations of infected cells with a different half-life, as proposed by Shekhtman et al.[33] in a series of 18 patients treated with BLV monotherapy. Interestingly, the theoretical framework of this model supported the use of drugs blocking viral production, such as Peg-IFN, to potentiate BLV antiviral efficacy and accelerate viral kinetics. Although this prediction is corroborated by our results, our model nonetheless emphasizes the large inter-patient variability in the response to both drugs and the risk of treatment dropout, both factors that may constitute an important hurdle to viral eradication in real life. With a substantial proportion of patients that may be non-responders despite prolonged therapy, it will be critical that future models identify baseline and on-treatment predictors of non-response.

Undoubtedly, the main limitation of these results is that it relies on two observational studies, and not on a randomized clinical trial. Although baseline characteristics were largely similar between the two treatment groups (except for platelet counts), a treatment bias is possible. The direction of this bias is difficult to anticipate, and could lead to overestimate the effect of Peg-IFN (if the combination was given to patients in better condition, as hinted by the higher level of platelet counts at baseline) or underestimate it (if treatment was given in priority to more severe patients). Also, the analysis of the synergism between BLV and Peg-IFN is hampered by the absence of a control group receiving Peg-IFN only. Consequently, our model cannot identify an effect of BLV on other viral kinetic parameters, making it possible that the effects of BLV could be underestimated. Another important limitation of our results is the use of the cure boundary[34] to predict the time needed to clear the last virus particle. This concept was successfully developed in HCV to guide treatment duration. However, the dynamics of HDV in the long term, and especially below the limit of quantification, could be more complex than HCV, and depend on unknown interaction with HBV proteins expressed from both cccDNA and integrated HBV DNA.

Finally, our results suggest that the addition of Peg-IFN may have some virological benefit. However, given the poor tolerance and the high variability in the response to Peg-IFN, the impact of a more rapid virological response on the liver functions and more generally on the clinical outcome will need to be evaluated. In that direction, larger prospective randomized studies will be needed to evaluate the predictive factors of response or non-response (Figs. S14 and S15).[42]

In summary, our analysis shows a clear effect of Peg-IFN in enhancing viral kinetics in patients treated with BLV. Randomized clinical trials are warranted to assess the virological and clinical benefit of the combination.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2024.101070.

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Author names in bold designate shared co-first authorship

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Supplemental information

Effect of Peg-IFN on the viral kinetics of patients with HDV infection treated with bulevirtide

Selma El Messaoudi, Ségolène Brichler, Claire Fougerou-Leurent, Emmanuel Gordien, Athenaïs Gerber, Amal Kortebi, Garance Lagadic, Miroslava Subic-Levrero, Sophie Metivier, Stanislas Pol, Anne Minello, Vlad Ratziu, Vincent Leroy, Philippe Mathurin, Laurent Alric, Fatoumata Coulibaly, Jean-Michel Pawlotsky, Fabien Zoulim, Victor de Lédinghen, Jérémie Guedj, and the ANRS HD EP01 BULEDELTA Study Group

Effect of Peg-IFN on the viral kinetics of HDV infected patients treated with bulevirtide

Supplementary materials

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73

74

75
76

Additional text

77

78

79   *1. Calibration curves*

80

81   We identified heterogeneity in the units informed in the data, with some observations informed in
82   cp/mL, and others in IU/mL. We developed calibration curves to homogenize the concentrations.

83   *Methods*

84   The French National Reference Center for HDV organizes each year a program for external quality
85   assessment of HDV RNA quantification. Therefore, the same samples are measured using both
86   home-made techniques (in cp/mL), and the reference technique (in IU/mL). (**Figure S9**). Those data
87   were used to derive linear regression curves.

88

89   For each center, linear regression (relationship between the concentration measured with the
90   reference technique and in each center (home-made technique) to predict the concentration in
91   IU/mL.

92    $C_{Avicenne} = aC_{centre}^{i} + b$

93   with $C_{Avicenne}$ the concentration measured in Avicenne, C_{centre}^{i} : concentration measured in center i,
94   a the slope, b the intercept

95   The linear regression curves are represented in **Figure S10.**

96
97   *2. Survival analysis*

98   The probability of treatment discontinuation (**Figure S11**) was estimated for each treatment
99   independently with an exponential model as follow :

100
$$S(t) = exp(-\lambda t)$$

101   The event was defined as a definitive cessation of treatment.

102

103   *2. Model prediction : results of the intention-to-treat scenario*
104

105   In the intention-to-treat scenario, where the risk of treatment discontinuation of both BLV and Peg-
106   IFN is accounted for, these rates were slightly lower. After 48 weeks, we predicted a virological
107   response of 48.2% (PI 95% = [41.2 ; 56.2]) and 78.3% (PI 95% = [66.7 ; 88.4]) in patients treated
108   with BLV and BLV+Peg-IFN, respectively (**Figure 4B**).
109   After 144 weeks of treatment, these values were equal to 60.5% (95% PI = [54.4 ; 70.2]) and 85.5%
110   (95% PI = [76.8 ; 92.8]), respectively, and 79.7% (95% PI = [71.0 ; 88.4]) for the
111   intermediate treatment strategy.
112   The rates of combined response after 48 weeks were predicted to be 19.3% (PI 95% = [14.0 ; 27.42])
113   and 30.4% (PI 95% = [20.3 ; 42.0]), with BLV monotherapy and BLV+Peg-IFN, respectively and
114   increased to 24.6% (PI 95% = [17.5 ; 33.3]), 34.8% (PI 95% = [24.6 ; 44.9]) and 31.9% (PI 95% =
115   [21.7 ; 43.5]) with BLV monotherapy, BLV+Peg-IFN, and the intermediate treatment strategy,
116   respectively.
117   Regarding the rates of undetectability, after 48 weeks we predicted rates of 30.7% (PI 95% = [22.8
118   ; 37.7]) and 55.8% (PI 95% = [43.5 ; 69.6]) with BLV monotherapy or BLV + Peg-IFN, respectively
119   and after 144 weeks of treatment, those rates increased to 50% (PI 95% = [41.3 ; 58.7]), 73.9% (PI
120   95% = [63.8 ; 85.5]) and 62.3% (PI 95% = [52.2 ; 76.8]) with BLV monotherapy, BLV+Peg-IFN and
121   the intermediate treatment strategy, respectively.
122   The rate of viral cure would drop to 7.0% (95% PI = 2.6 ; 12.3]) and 15.9% (95% PI = [7.2 ; 24.6]) in
123   patients treated with BLV and BLV+Peg-IFN, respectively, after 48 weeks. After 96 weeks, these
124   rates were equal to 19.7% (95% PI = 14.0 ; 36.3]), 36.2% (95% PI = [24.6 ; 47.9]) and 23.2 (95% PI
125   = [14.5 ; 34.8]) , for the monotherapy, the combination therapy and the intermediate treatment
126   strategy, respectively.
127   The rate of viral cure would drop to 7.0% (95% PI = 2.6 ; 12.3]) and 15.9% (95% PI = [7.2 ; 24.6]) in
128   patients treated with BLV and BLV+Peg-IFN, respectively, after 48 weeks. After 96 weeks, these
129   rates were equal to 19.7% (95% PI = 14.0 ; 36.3]), 36.2% (95% PI = [24.6 ; 47.9]) and 23.2 (95% PI
130   = [14.5 ; 34.8]) , for the monotherapy, the combination therapy and the intermediate treatment
131   strategy, respectively.

132

133

134   *2. Model code*
135   DESCRIPTION: Neumann-Lam model (Neumann et al., Science, 282, 1998)

136

137   [LONGITUDINAL]

```
138   input = {beta_log, delta, p, c, eps_beta_BLV, eps_p_PEG, An, A0, ca,  V0_log, tlag, lambda_BLV,
139   lambda_PEG, Start_PEG, End_PEG_1, Restart_PEG_1, End_PEG_2,  Restart_PEG_2,
140   End_PEG_3, First_stop_BLV, Restart_BLV_1, End_BLV_2, Restart_BLV_2}
141   Start_PEG={use=regressor}
142    End_PEG_1={use=regressor}
143   Restart_PEG_1={use=regressor}
144   End_PEG_2={use=regressor}
145   Restart_PEG_2={use=regressor}
146   End_PEG_3={use=regressor}
147   First_stop_BLV={use=regressor}
148   Restart_BLV_1={use=regressor}
149   End_BLV_2={use=regressor}
150   Restart_BLV_2={use=regressor}
151
152   EQUATION:
153   ; Initial conditions
154   t0     = 0
155   beta=10^beta_log
156   V0= 10^V0_log
157   T0= (c*delta)/(beta*p)
158   IC_0  = (beta*V0*T0)/delta
159   VL_0 = V0
160
161
162   ; Before IFN both eta and epsilon equal 0. Once therapy is initiated, both are >0
163   ; inhibition before and after the end of treatment
164
165   ; Dates PEG
166
167   if t <0
168   BLV =0
169   end
170
171   if  t >= First_stop_BLV & t < Restart_BLV_1
172   BLV =0
173   end
174
```

```
175    if t >= End_BLV_2 & t < Restart_BLV_2
176    BLV =0
177    end
178    if t >=0 & t < First_stop_BLV
179    BLV=1
180    end
181
182    if t >=Restart_BLV_1 & t < End_BLV_2
183    BLV=1
184    end
185
186    if t >=Restart_BLV_2
187    BLV=1
188    end
189
190    ; Dates PEG
191    if t < Start_PEG + tlag
192    PEG =0
193    end
194
195    if  t >= End_PEG_1 & t < Restart_PEG_1
196    PEG =0
197    end
198
199    if t >= End_PEG_2 & t < Restart_BLV_2
200    PEG =0
201    end
202
203    if t >= End_PEG_3
204    PEG =0
205    end
206
207    if t >=Start_PEG + tlag & t < End_PEG_1
208    PEG =1
209    end
210
211    if t >=Restart_PEG_1 + tlag & t < End_PEG_2
```

```
212    PEG=1
213    end
214
215    if t >=Restart_PEG_2 + tlag & t < End_PEG_3
216    PEG=1
217    end
218
219    if BLV==0 & PEG==0
220     eps_beta = 0
221     eps_p = 0
222
223    elseif BLV==1 & PEG==0
224     eps_beta = eps_beta_BLV
225     eps_p = 0
226    elseif   BLV==0 & PEG==1
227     eps_beta = 0
228    eps_p= eps_p_PEG
229    else
230    eps_beta =eps_beta_BLV
231    eps_p= eps_p_PEG
232    end
233
234    ALT = An*(1 - (A0-An)/(An*(ca-delta))*(delta*exp(-ca*t)-exp(-delta*t)))
235
236    ; Viral dynamic model
237    ddt_IC  = beta*(1-eps_beta)*T0*VL - delta*IC
238    ddt_VL = p*(1-eps_p)*IC - c*VL
239
240    LVL = log10(max(VL,-3)) ; to have LVL positive
241
242    ; Survival model
243    haz_BLV  = lambda_BLV
244    haz_PEG  = lambda_PEG
245
246    DEFINITION:
247    Event_BLV= {type=event, maxEventNumber=1, hazard=haz_BLV}
248    Event_PEG= {type=event, maxEventNumber=1, hazard=haz_PEG}
```

249

250 OUTPUT:

251 output ={LVL, ALT, Event_BLV, Event_PEG}

252

253 Figures

254 *1. Observations and treatment assigned*
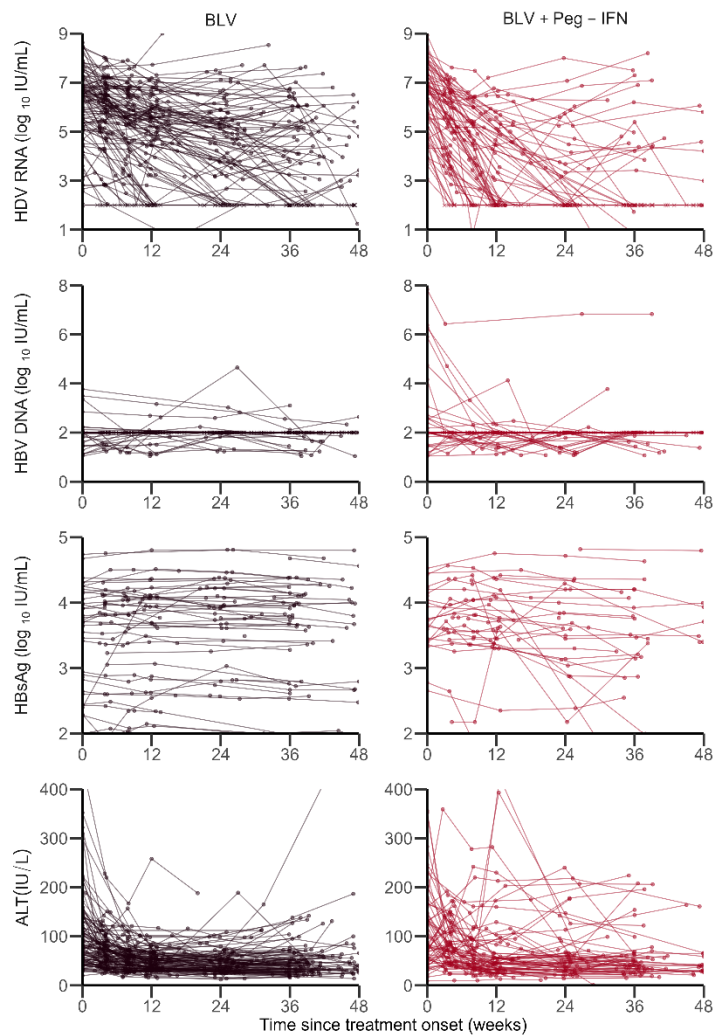
Figure S1: Dynamics of HDV RNA, HBV DNA, HBsAg and ALT during treatment in the group treated with bulevirtide alone (BLV) or in combination with Peg-IFN (BLV+Peg-IFN).

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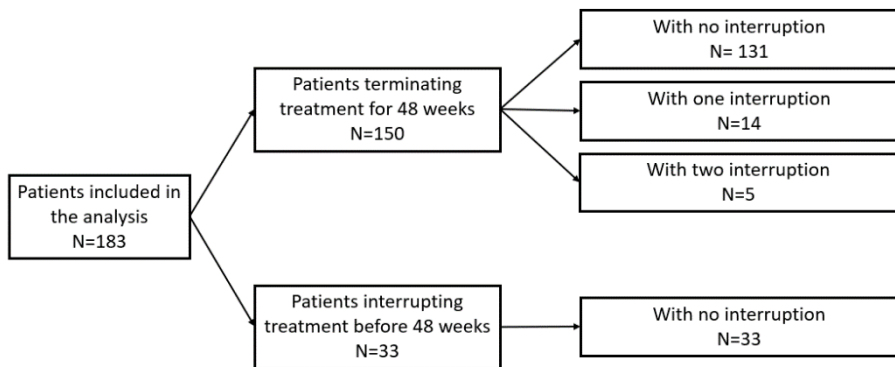
277 **Figure S2**: Workflow of the number of patients interrupting treatment during the analysis.

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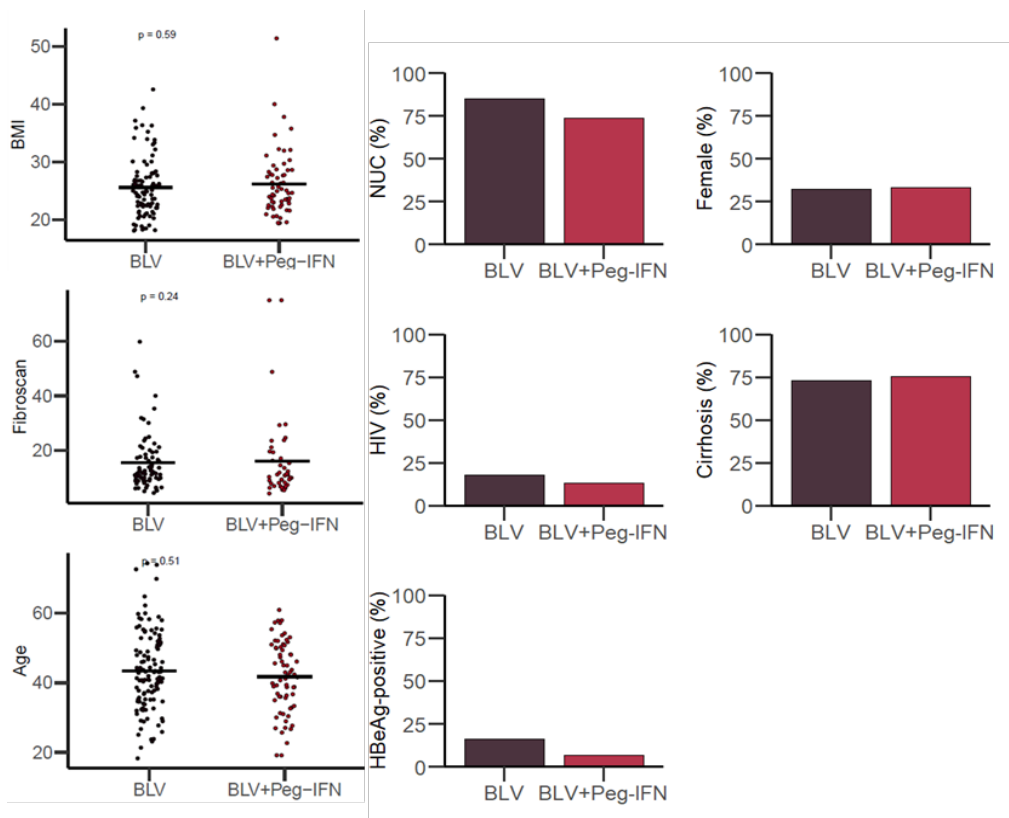
290 **Figure S3:** Patients characteristics versus treatment received at baseline. Black represents the group treated
291 with monotherapy and red represents the group treated with the combination.

292

293

2. Doses of Peg-IFN

The dose of Peg-IFN was available in 46 patients. Among them, the dose was adjusted throughout the analysis in 10 patients (8 for whom the dose was reduced, and 2 for whom the dose was increased.) For 3 patients, the dose was reduced twice.
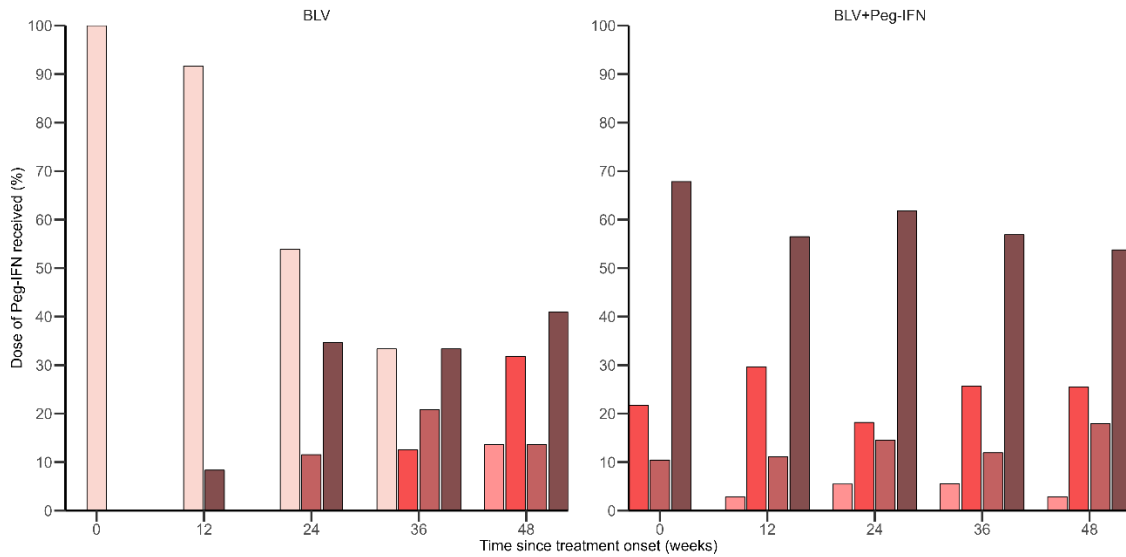
Figure S4: Percentage of doses of Peg-IFN available during the analysis. Each level of the gradient of reds correspond to a dose (0, 45, 90, 180), the higher the dose, the darker the red.

312 *3. Treatment response*

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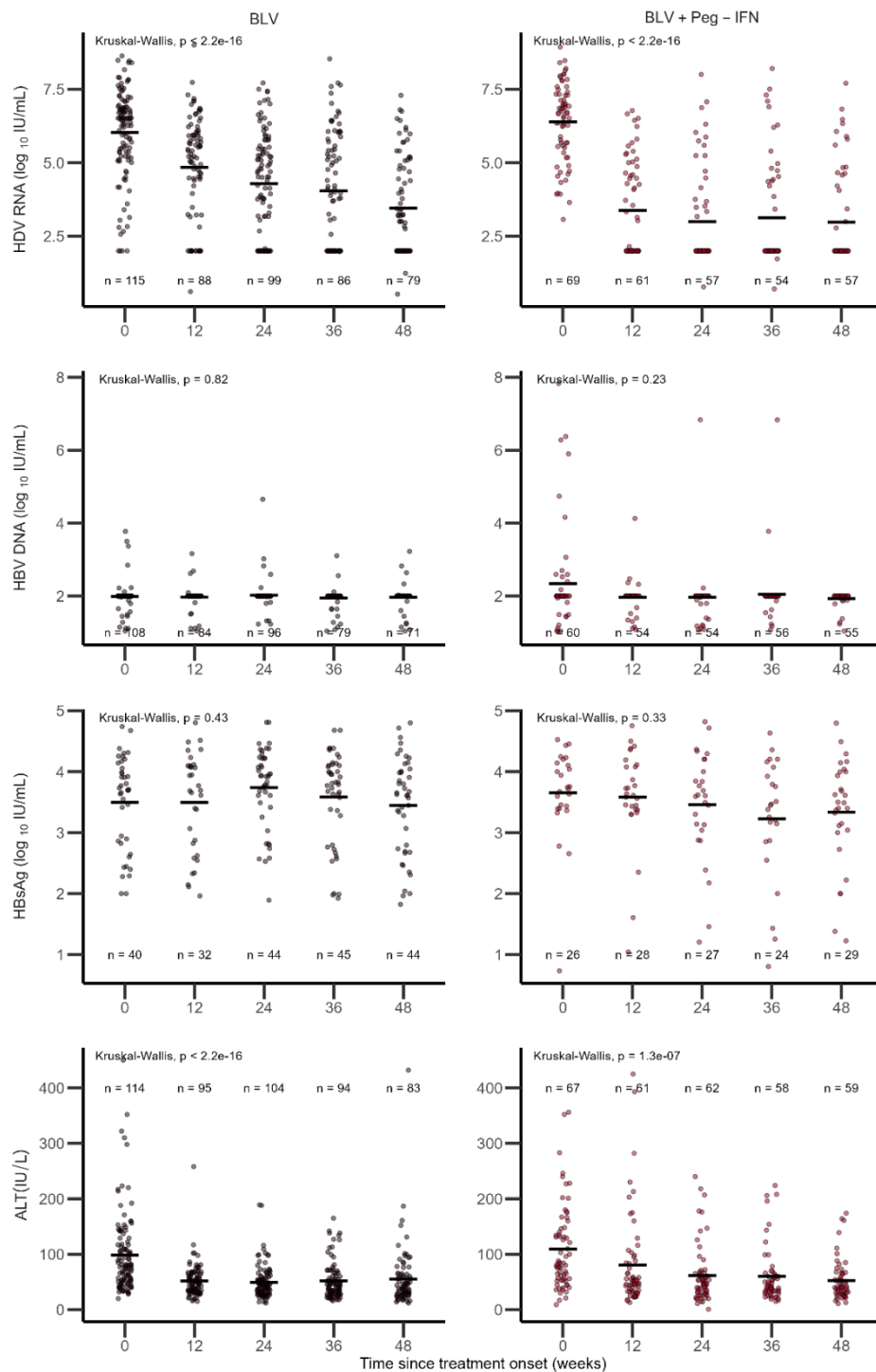
332 **Figure S5:** Virologic and biochemical decline kinetics in each group. The plain line represents the
333 median,n represent the number of observations
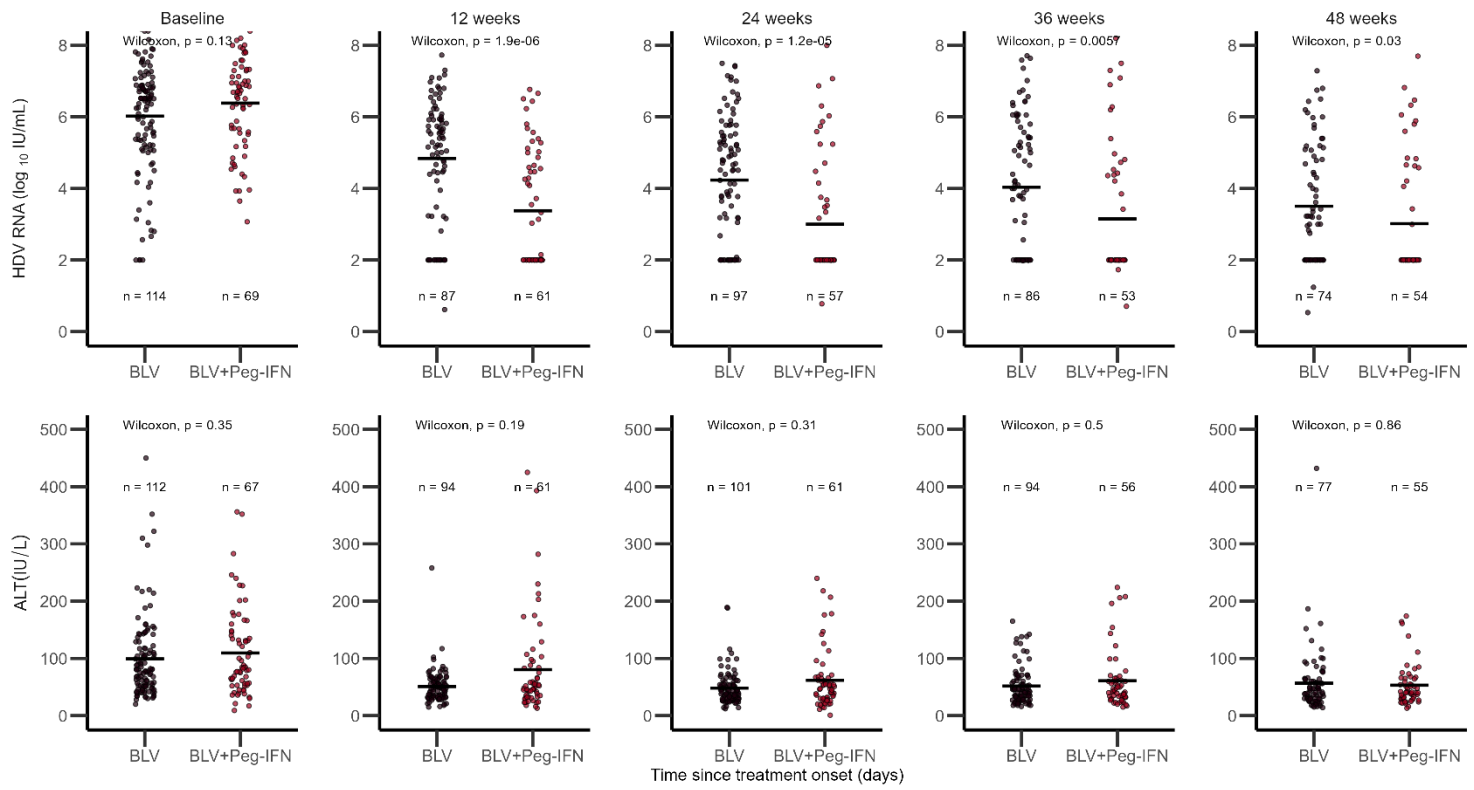
Figure S6: Distribution of the observed HDV RNA and ALT levels across the study in groups Bulevirtide (BLV) and bulevirite+Peg-IFN (BLV+Peg-IFN). The plain line represents the median concentration, n represent the number of observations.
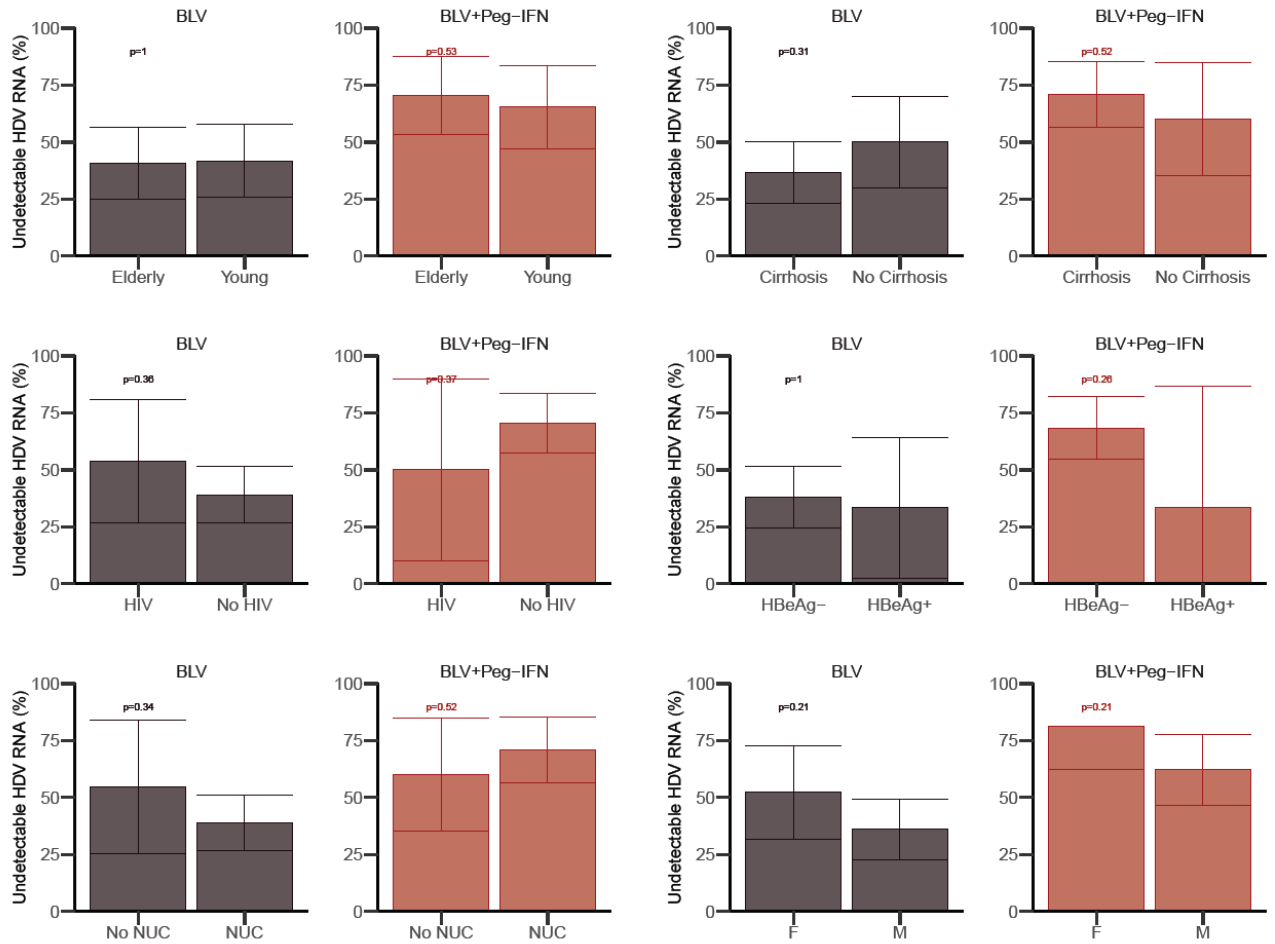
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340 **Figure S7 :** Percentage of undetectable HDV RNA in each group according to baseline covariates. The
341 errorbars correspond to the 95% confidence interval of a binomial law.

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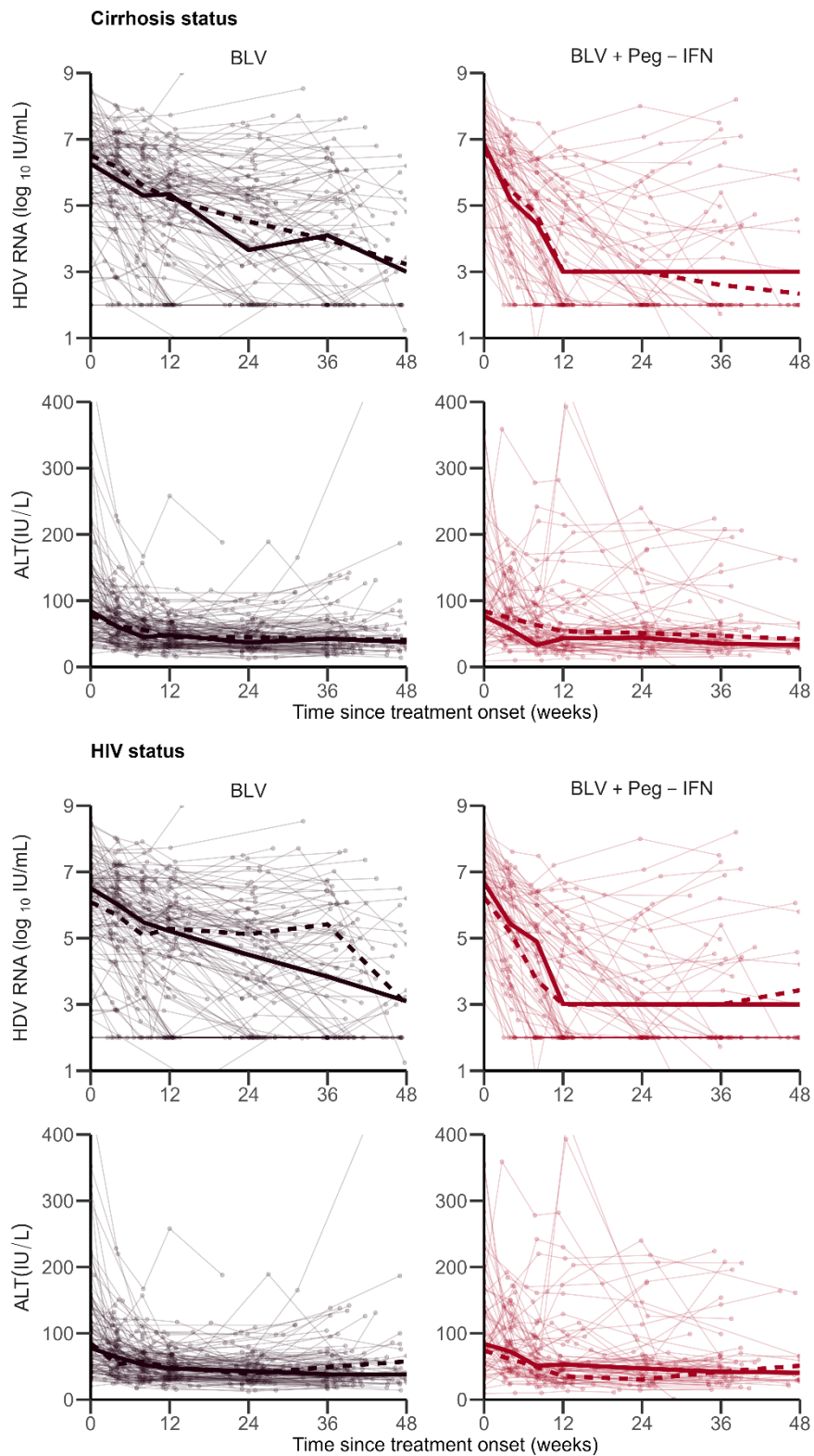362
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375 **Figure S8 :** HDV RNA and ALT kinetics observed in each group. The large line correspond to the median
376 observed in top : cirrhotic patients (plain line) versus non-cirrhotic patients (dashed line) ; bottom : HIV patients
377 (plain line) versus non-HIV patients (dashed line).

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4. Calibration curves
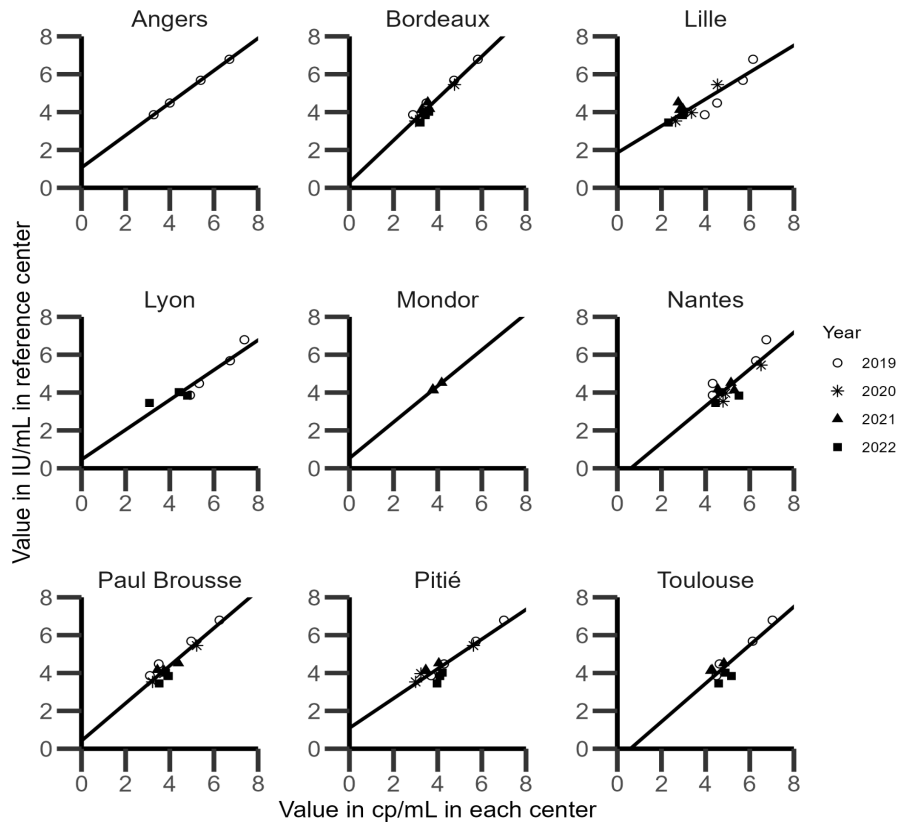

Figure S9: Measurements of the samples used to derive the linear regression, with their value with "home-made" techniques and the reference technique.
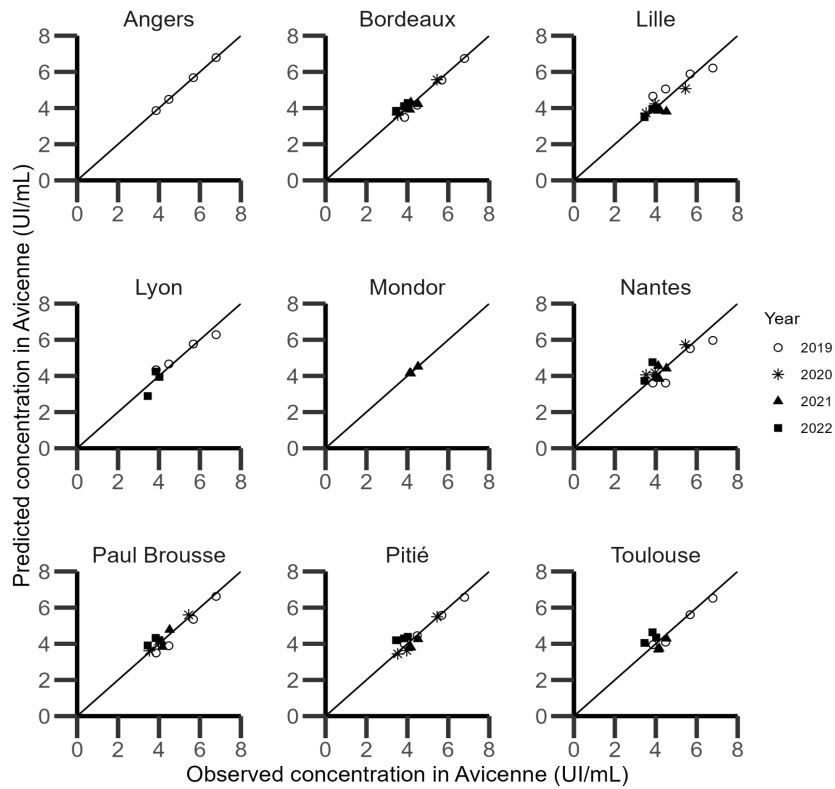
423   **Figure S10**: Linear regression curves allowing to derive concentrations from the reference technique.
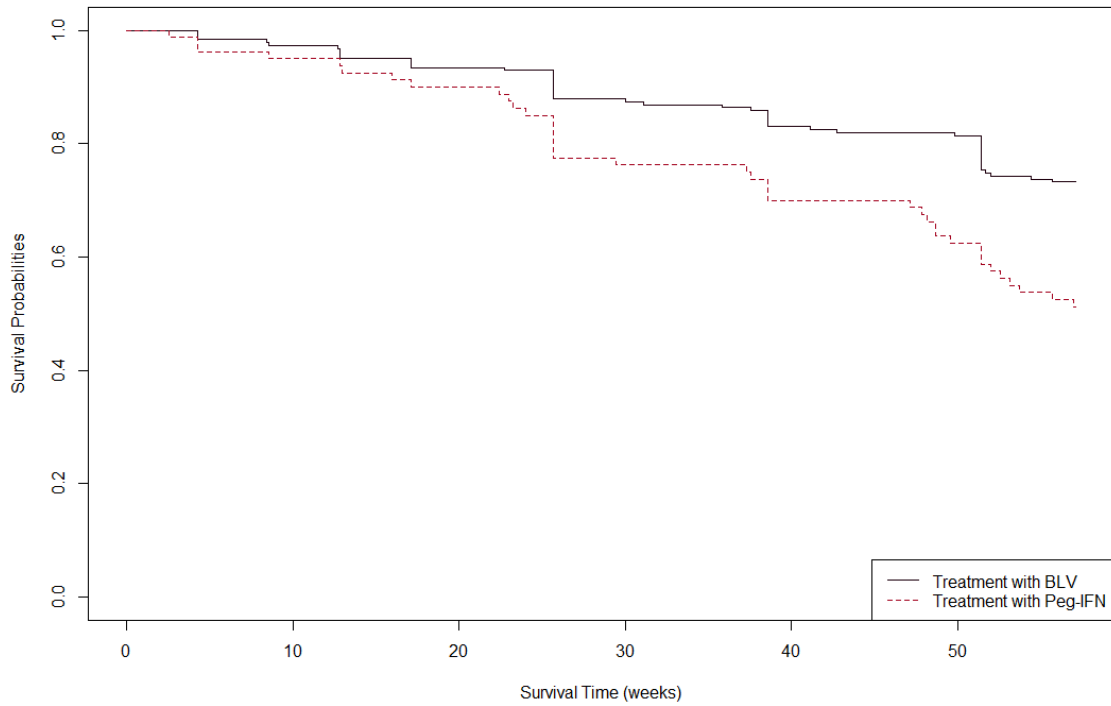
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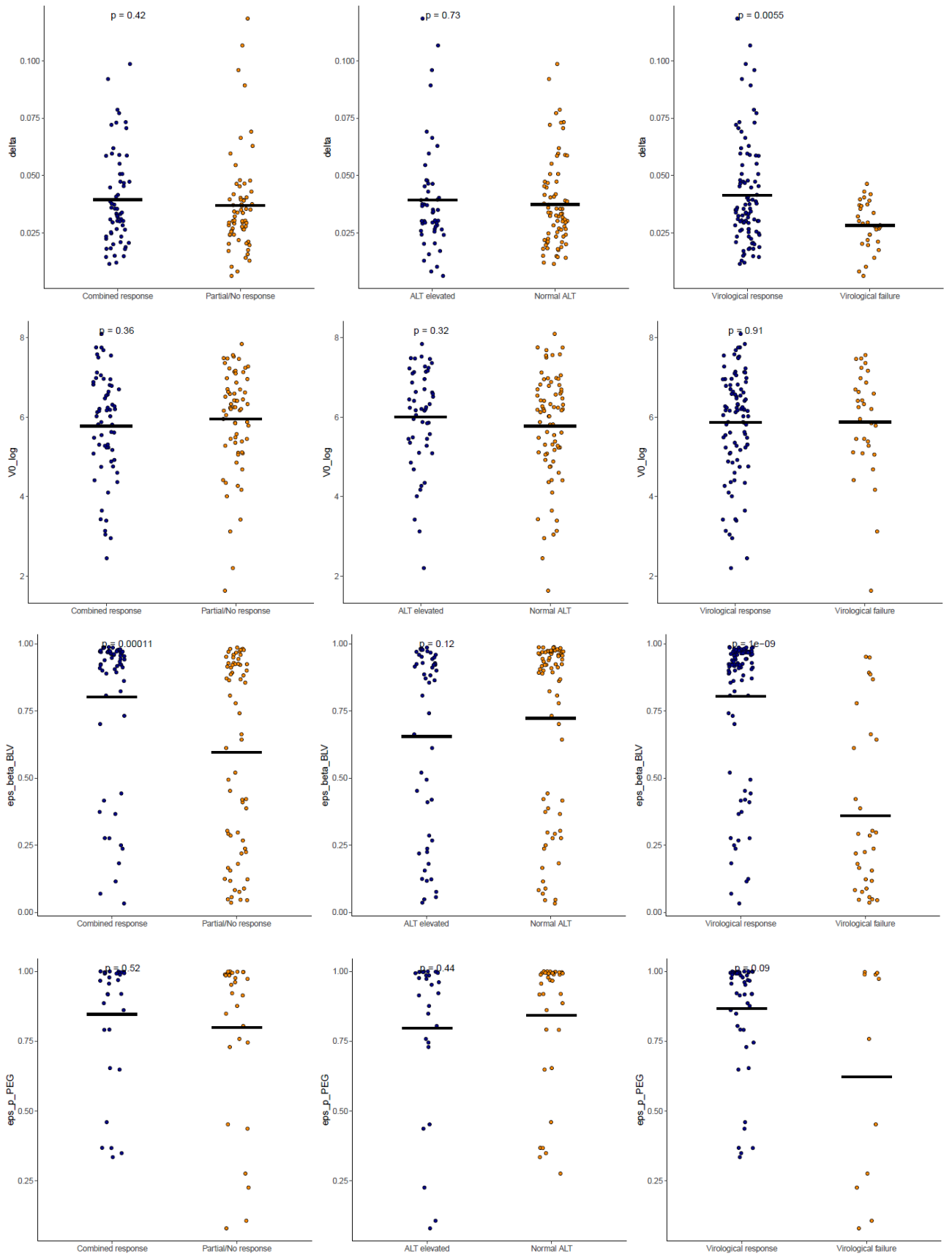430   *5. Survival analysis : Probability of stopping treatment*

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432   **Figure S11**: Kaplan-Meyer curves on the survival probability of treatment cessation.
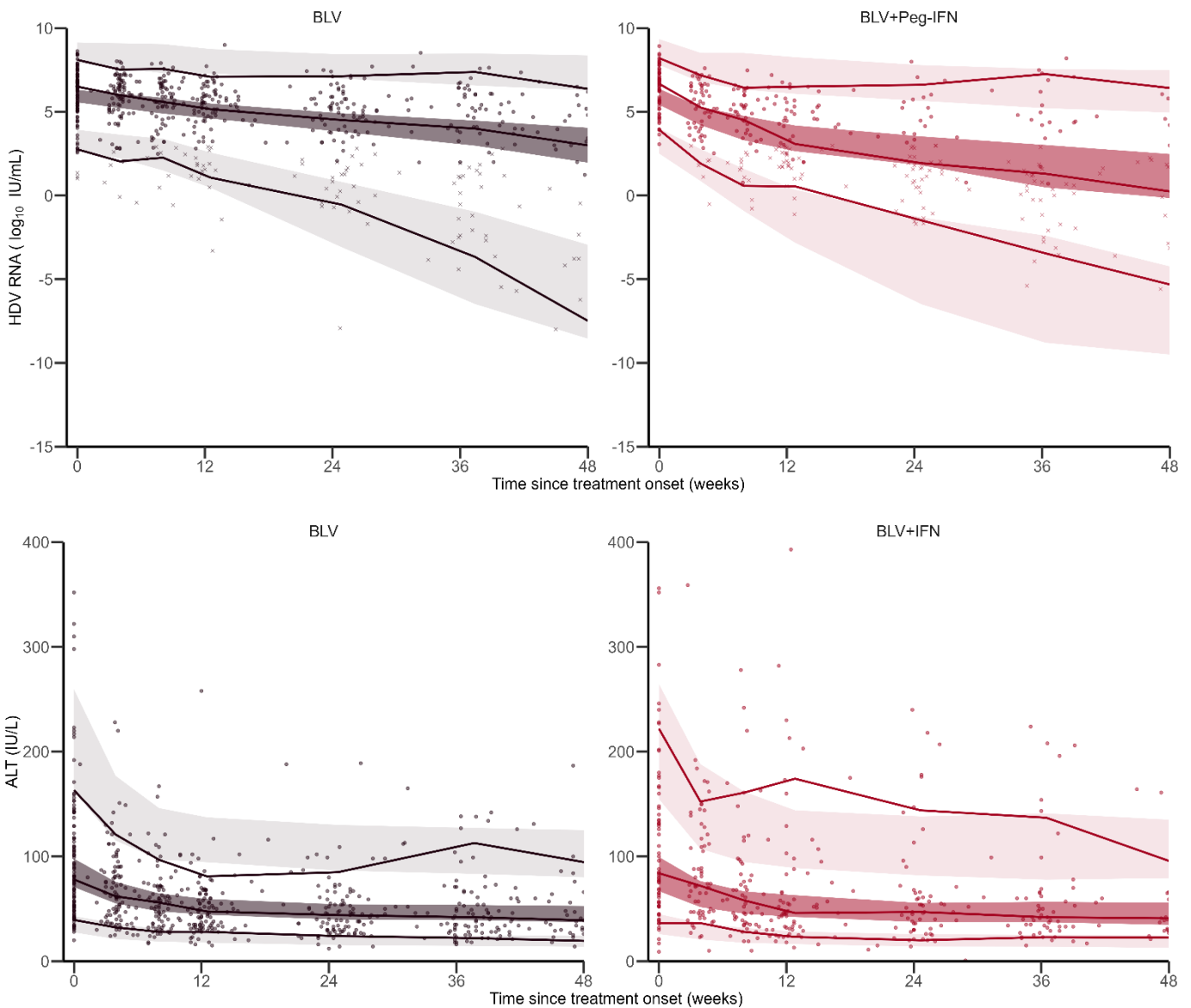
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438 **Figure S12 :** Distribution of parameters according to the virological, biochemical or combined response of

439 the patients.

441   The model was evaluated using visual predictive checks (VPCs) and individual fits (Figure S2).

442   **Figure S13**: Visual predictive checks (VPCs) stratified on arms Bulevirtide monotherapy (BLV) or

443   in combination with Peg-IFN. Dots represent observed values, plain lines represent the 5th, 50th

444   and 95th empirical percentiles on observed data. Shaded areas represent the 95% prediction

445   interval around the corresponding percentiles.

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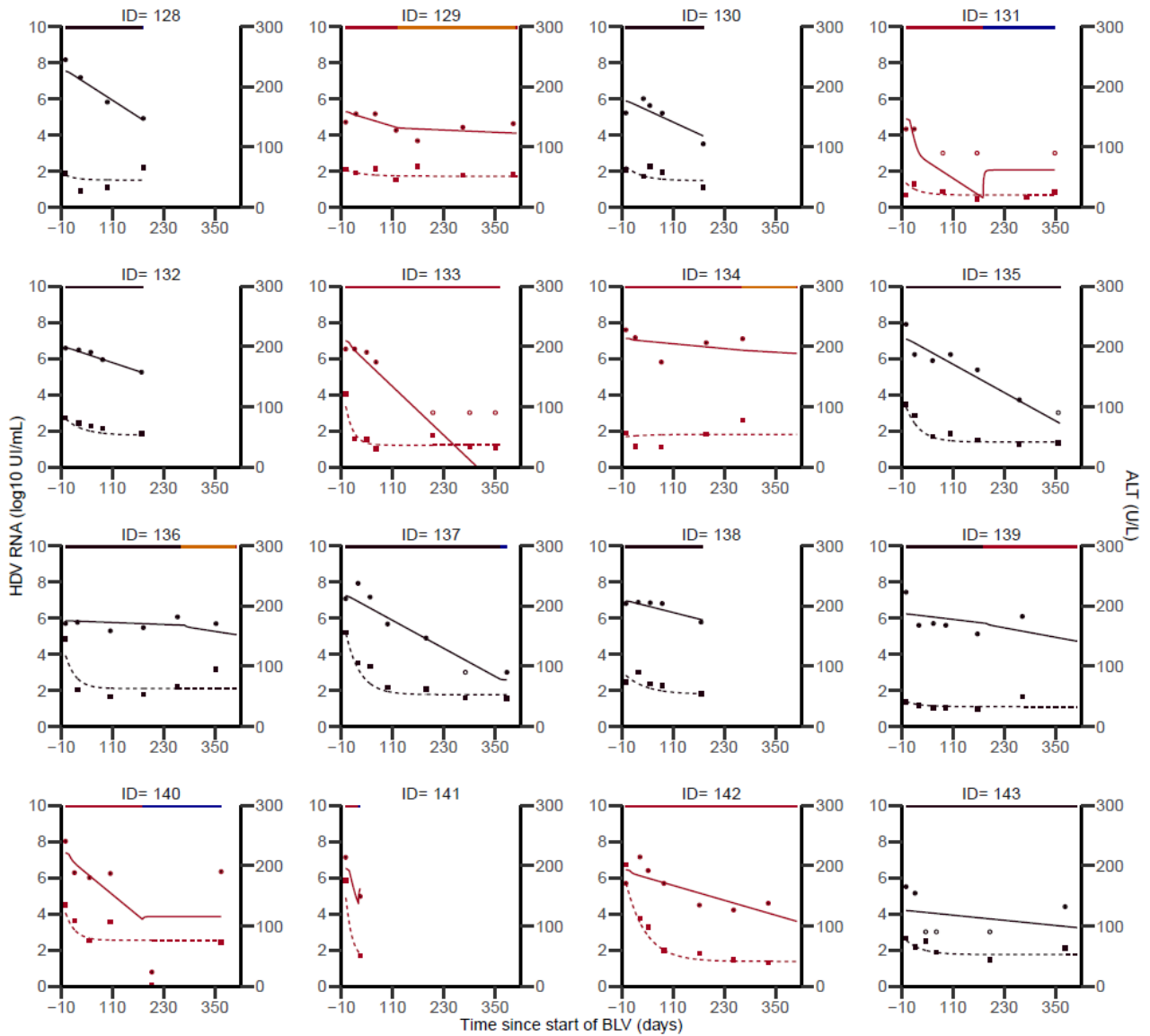
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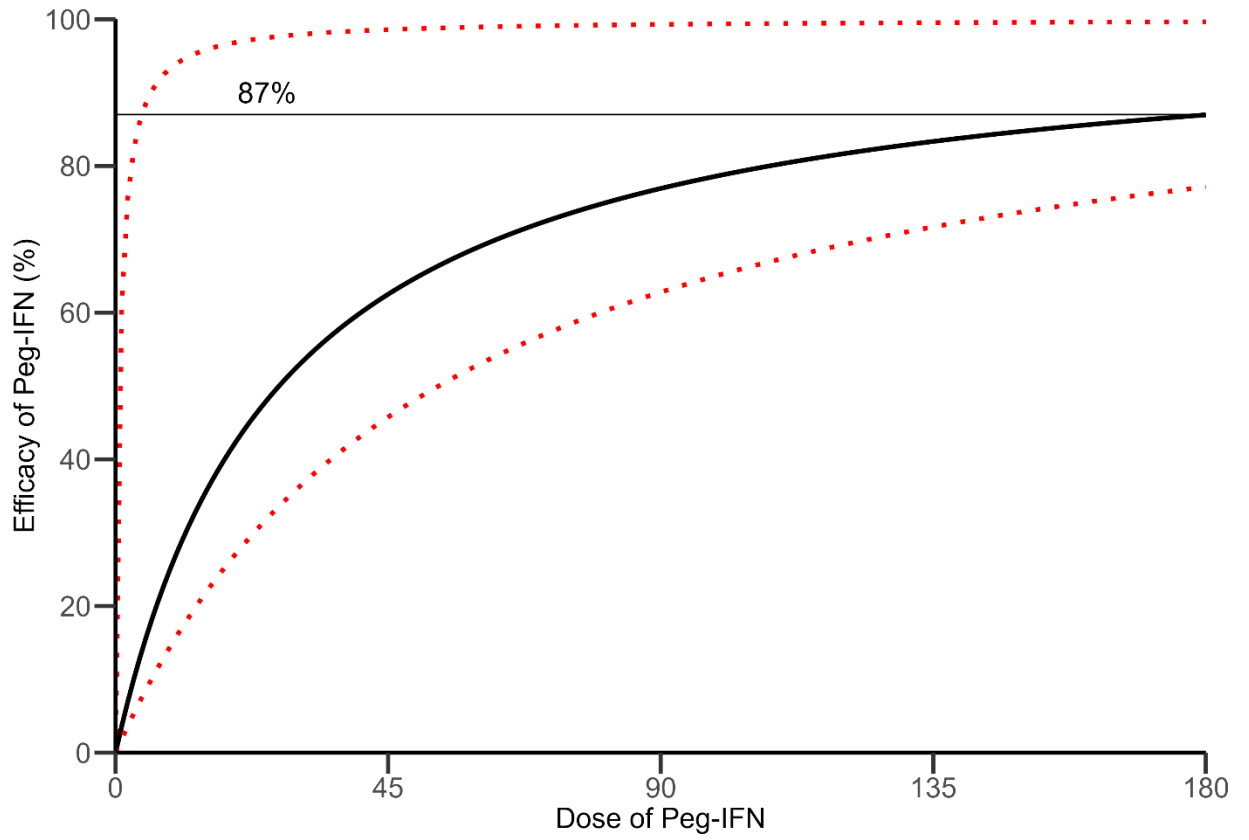449   **Figure S14**: Individual predictions of HDV RNA (plain line) and ALT (dotted lines) in patients in the

450   group Bulevirtide (black lines) or bulevirtide+Peg-IFN (red lines). The top plain line indicates the

451   treatment received at each time (Red, Black, orange and blue for BLV+Peg-IFN, BLV only, Peg-

452   IFN only and off-treatment, respectively).

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Figure S15: Relationship between the dose of Peg-IFN and the efficacy on blocking viral production in the sensitivity analysis taking into account the dose.

471   Tables

472   *1. Limit of quantification*

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474   **Table S1 :** Summary of the number of observation associated with the different limit of quantifications
475   (LOQ) for HDV RNA available in our data.

Limit of quantification (IU/mL)	Number of observations (n)
2	117
2.2	13
2.4	3
2.48	3
2.6	4
3	141

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483   *2. Estimation of treatment effect*

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485   **Table S2** : Results of the model building of drug inclusion

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		BIC	-2 LL
Identifying the main effect of Peg-IFN	Effect of BLV on β only	12567.07	12494.13
	Effect of BLV on β + effect of Peg-IFN on β	12556.02	12472.67
	Effect of BLV on β + effect of Peg-IFN on δ	12546.71	12463.36
	Effect of BLV on β + effect of Peg-IFN on p	12481.69	12398.34
Exploring additional effects of Peg-IFN	Effect of BLV on β + effect of Peg-IFN on p + effect of Peg-IFN on β	12480.88	12397.11
	Effect of BLV on β + effect of Peg-IFN on p + effect of Peg-IFN on δ	12490.00	12396.23

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505 *3. Sensitivity analysis taking into account the dose of Peg-IFN*

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507 **Table S3 :** Parameters estimated in the sensitivity analysis accounting for the dose of Peg-IFN.

		Parameter estimate (RSE%)	SD of the random effect (RSE%)
Disease parameters			
δ	Loss rate of infected cells (d^{-1})	$2.78\ 10^{-2}$ (14.8)	0.814 (15.7)
V_0	Number of virions at baseline (log IU/mL)	5.84 (2.15)	1.39 (6.83)
c_a	ALT clearance (d^{-1})	2.38 (42.8)	0.535 (101)
A_0	ALT value at baseline (U/L)	123 (21.1)	0.725 (8.9)
$A_{co\text{-}young\ male}$	ALT value in absence of infection in young males (IU/L)	45.4 (9.01)	0.528 (6.93)
$A_{co\text{-}elderly}$	ALT value in absence of infection in elderly males (IU/L)	58.4 (9.35)	
$A_{co\text{-}Females}$	ALT value in absence of infection in young females (IU/L)	35.9 (10.1)	
Drug effects			
$\varepsilon_{\beta}^{BLV}$	Effect of BLV on blocking infection	0.933 (10.7)	5.25 (40.0)
$ED90^{PEG}$	Effect of Peg-IFN on blocking viral production	243 (124)	5.33 (32.8)
Residual error model			
$a_{HDV\ RNA}$	Additive residual error on HDV RNA (log$_{10}$ IU/mL)	0.754 (3.61)	
b_{ALT}	Proportional residual error on ALT	0.310 (3.14)	

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516 *4. Model prediction : comparison to MY204 study*

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518 **Table S4** : Comparison of the prediction of the model in the intention-to-treat scenario versus MYR204.

519 The simulated LOQ was set to 50 IU/mL.

	BLV+ PEG for 1y then BLV		*BLV only (10 mg in MYR204, 2mg in the predictions)*	
	Median predictions from the model	*Myr 204*	*Median predictions from the model*	*Myr 204*
Undetectable VL EoT	*55%*	*44% (CI = [30 ; 60])*	*44%*	*22% (CI=[15 ; 38])*
Undetectable VL 24 FU (or Viral cure)	*25%*	*32% (IC=[20 ; 45])*	*20%*	*12% (CI=[5 ; 25])*
ALT normalisation	*40%*	*42% (IC=[30 ; 58])*	*39%*	*30% (CI=[20 ; 45])*

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