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

Supplementary materials

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

Table of contents

List of investigators

Sophie Metivier[1], Victor de Ledinghen[2], Stanislas Pol[3], Anne Minello[4], Vlad Ratziu[5], Vincent Leroy[6], Philippe Mathurin[7], Laurent Alric[8], Nathalie Ganne[9], Fabien Zoulim[10], Véronique Loustaud-Ratti[11], Dominique Roulot[9], Karine Lacombe[12], Caroline Lascoux-Combe[12], Georges-Philippe Pageaux[13], Isabelle Rosa[14], Caroline Jezequel[15], Jerôme Dumortier[16], Ghassan Riachi[17], Olivier Chazaouilleres[12], Dulce Alfaiate[18], Dider Samuel[19], Xavier Causse[20], Armando Abergel[21], Julie Chas[22], Anne Gervais[23], Marc Bourlière[24], Louis d'Alteroche[25]

1. Department of Hepatology, CHU Rangueil, Toulouse, France
2. Centre d'Investigation de la Fibrose Hépatique, Bordeaux University Hospital, Pessac, France; INSERM U1312, Bordeaux University, Bordeaux, France.
3. Department of Hepatology, Hôpital Cochin, AP-HP, Université Paris-René Descartes, INSERM U1016, Paris, France.
4. Department of Hepatology and Gastroenterology, University hospital Dijon, INSERM UMR 1231, France.
5. Sorbonne Université, Assistance Publique-Hôpitaux de Paris, Hôpital Pitié Salpêtrière-, Institute of Cardiometabolism and Nutrition (ICAN), Paris, France.
6. Department of Hepatology and Gastroenterology, Centre Hospitalo-Universitaire, INSERM U1209, Université Grenoble Alpes, Grenoble, France.
7. Service des maladies de l'appareil digestif, Université Lille 2 and Inserm U795, France.
8. Department of Internal Medicine and Digestive Diseases, UMR-152, Toulouse III University, France.
9. Department of Hepatology, Hôpital Avicenne AP-HP, Bobigny, France
10. Department of Hepatology, Hospices Civils de Lyon, France.
11. Department of Hepatology ; Dupuytren Hospital, Limoges, France
12. Department of Infectious and Tropical Diseases, Saint-Antoine Hospital- APHP, Paris, France
13. Department of Hepatology, Saint Eloi Hospital, Montpellier, France .
14. Department of Hepatology, CHIC, Créteil, France.
15. Department of Hepatology, Pontchaillou Hospital, Rennes, France.
16. Department of Hepatology, Edouard Herriot Hospital, Lyon, France.
17. Department of Hepatology, Charles Nicolle Hospital, Rouen, France
18. Department of Infectious and Tropical Diseases, Hospices Civils de Lyon, France.
19. Department of Hepatology, Paul Brousse Hospital- APHP, Villejuif, France

20. Department of Hepatology, La Source Hospital, Orléans, France
21. Department of Hepatology, Estaing Hospital, Clermont-Ferrand, France
22. Department of Infectious and Tropical Diseases, Tenon Hospital- APHP, Paris, France
23. Department of Infectious and Tropical Diseases, Bichat Hospital- APHP, Paris, France
24. Department of Hepatology, Saint Joseph Hospital- APHP, Paris, France
25. Department of Hepatology, Trousseau Hospital- APHP, Paris, France

Additional text

1. Calibration curves

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

Methods

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

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

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

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

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

2. Survival analysis

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

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

The event was defined as a definitive cessation of treatment.

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

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. After 48 weeks, we predicted a virological response of 48.2% (PI 95% = [41.2 ; 56.2]) and 78.3% (PI 95% = [66.7 ; 88.4]) in patients treated with BLV and BLV+Peg-IFN, respectively (**Figure 4B**).
After 144 weeks of treatment, these values were equal to 60.5% (95% PI = [54.4 ; 70.2]) and 85.5% (95% PI = [76.8 ; 92.8]), respectively, and 79.7% (95% PI = [71.0 ; 88.4]) for the intermediate treatment strategy.
The rates of combined response after 48 weeks were predicted to be 19.3% (PI 95% = [14.0 ; 27.42]) and 30.4% (PI 95% = [20.3 ; 42.0]), with BLV monotherapy and BLV+Peg-IFN, respectively and increased to 24.6% (PI 95% = [17.5 ; 33.3]), 34.8% (PI 95% = [24.6 ; 44.9]) and 31.9% (PI 95% = [21.7 ; 43.5]) with BLV monotherapy, BLV+Peg-IFN, and the intermediate treatment strategy, respectively.
Regarding the rates of undetectability, after 48 weeks we predicted rates of 30.7% (PI 95% = [22.8 ; 37.7]) and 55.8% (PI 95% = [43.5 ; 69.6]) with BLV monotherapy or BLV + Peg-IFN, respectively and after 144 weeks of treatment, those rates increased to 50% (PI 95% = [41.3 ; 58.7]), 73.9% (PI 95% = [63.8 ; 85.5]) and 62.3% (PI 95% = [52.2 ; 76.8]) with BLV monotherapy, BLV+Peg-IFN and the intermediate treatment strategy, respectively.
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 patients treated with BLV and BLV+Peg-IFN, respectively, after 48 weeks. After 96 weeks, these 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 = [14.5 ; 34.8]) , for the monotherapy, the combination therapy and the intermediate treatment strategy, respectively.
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 patients treated with BLV and BLV+Peg-IFN, respectively, after 48 weeks. After 96 weeks, these 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 = [14.5 ; 34.8]) , for the monotherapy, the combination therapy and the intermediate treatment strategy, respectively.

2. Model code

DESCRIPTION: Neumann-Lam model (Neumann et al., Science, 282, 1998)

[LONGITUDINAL]

```
input = {beta_log, delta, p, c, eps_beta_BLV, eps_p_PEG, An, A0, ca,  V0_log, tlag, lambda_BLV,
lambda_PEG, Start_PEG, End_PEG_1, Restart_PEG_1, End_PEG_2,  Restart_PEG_2,
End_PEG_3, First_stop_BLV, Restart_BLV_1, End_BLV_2, Restart_BLV_2}
Start_PEG={use=regressor}
 End_PEG_1={use=regressor}
Restart_PEG_1={use=regressor}
End_PEG_2={use=regressor}
Restart_PEG_2={use=regressor}
End_PEG_3={use=regressor}
First_stop_BLV={use=regressor}
Restart_BLV_1={use=regressor}
End_BLV_2={use=regressor}
Restart_BLV_2={use=regressor}

EQUATION:
; Initial conditions
t0      = 0
beta=10^beta_log
V0= 10^V0_log
T0= (c*delta)/(beta*p)
IC_0  = (beta*V0*T0)/delta
VL_0 = V0


; Before IFN both eta and epsilon equal 0. Once therapy is initiated, both are >0
; inhibition before and after the end of treatment

; Dates PEG

if t <0
BLV =0
end

if  t >= First_stop_BLV & t < Restart_BLV_1
BLV =0
end

```

```
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}
```

OUTPUT:

output ={LVL, ALT, Event_BLV, Event_PEG}

Figures

1. Observations and treatment assigned

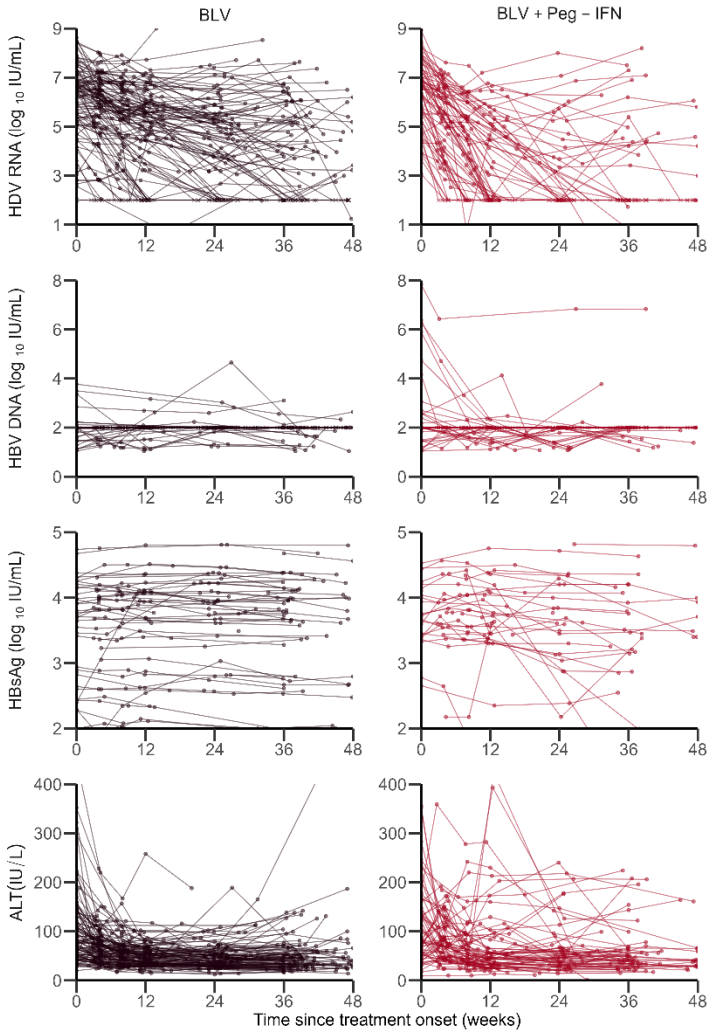


Figure S1: Dynamics of HDV RNA, HBV DNA, HBsAg and ALT during treatment in the group treated with bulevirtide alone (BLV) or in combination with Peg-IFN (BLV+Peg-IFN).

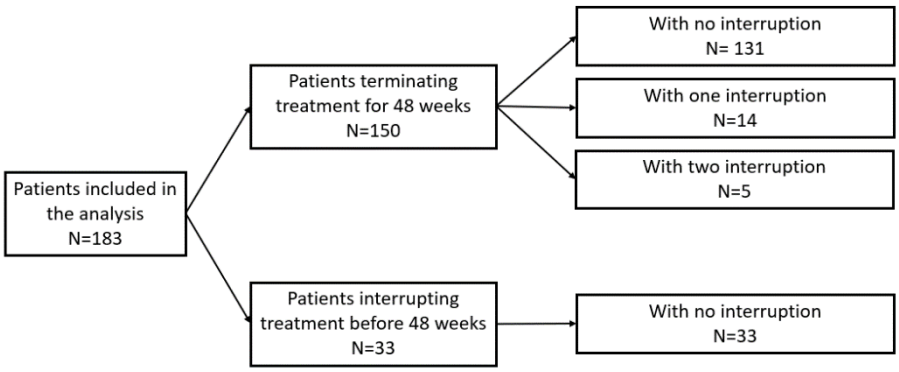


Figure S2: Workflow of the number of patients interrupting treatment during the analysis.

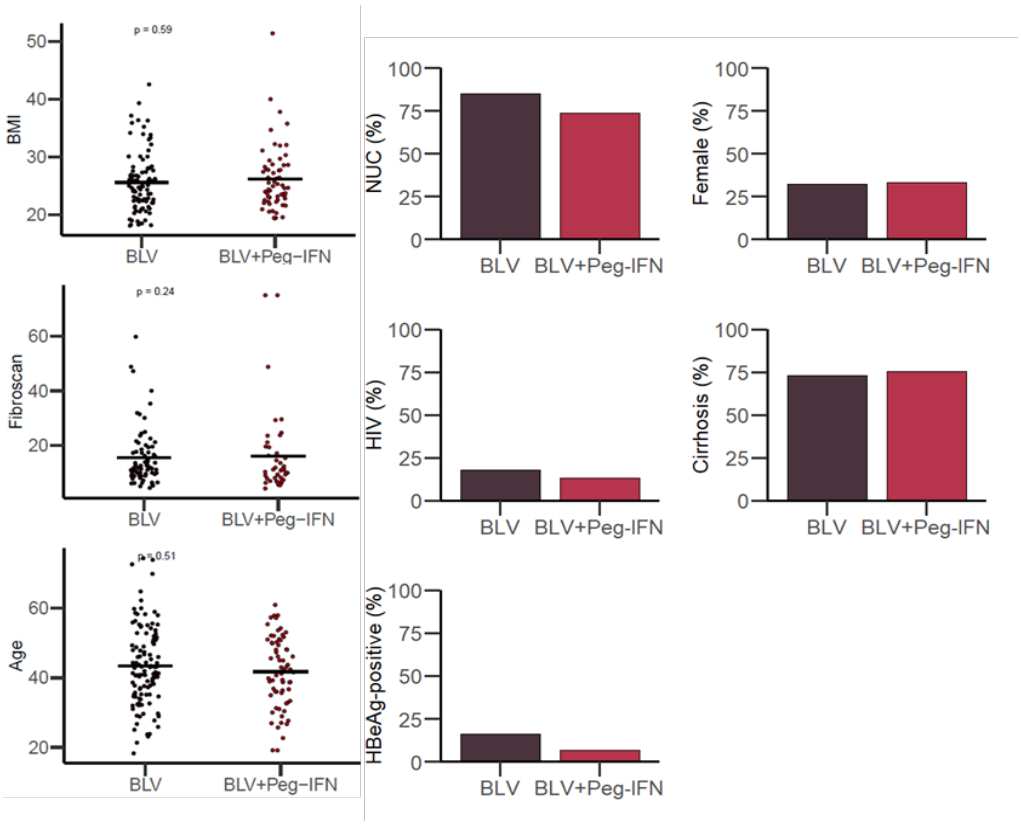


Figure S3: Patients characteristics versus treatment received at baseline. Black represents the group treated with monotherapy and red represents the group treated with the combination.

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.

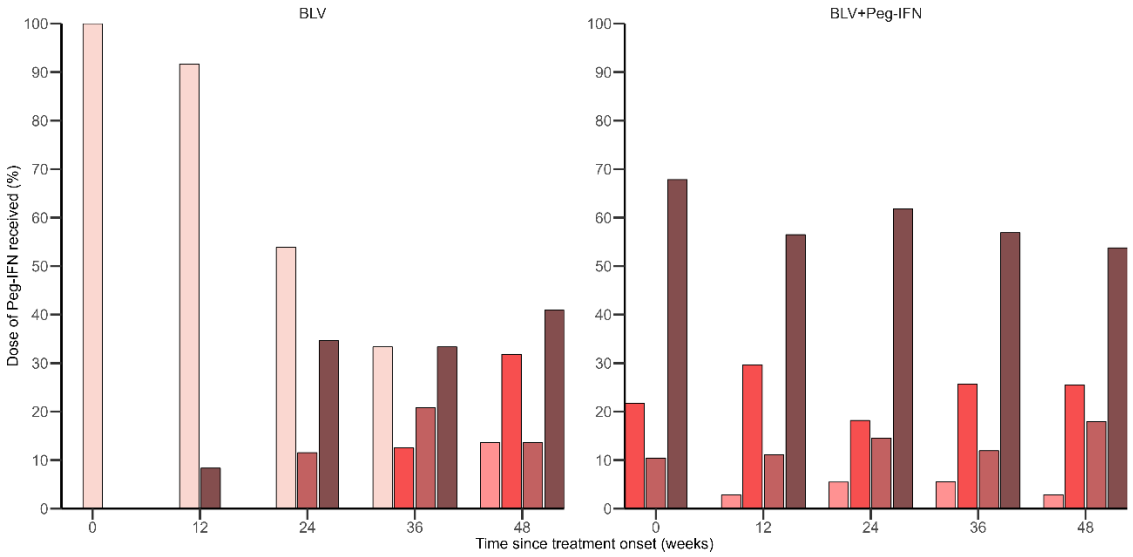


Figure S4: Percentage of doses of Peg-IFN available during the analysis. Each level of the gradient of reds correspond to a dose (0, 45, 90, 180), the higher the dose, the darker the red.

3. Treatment response

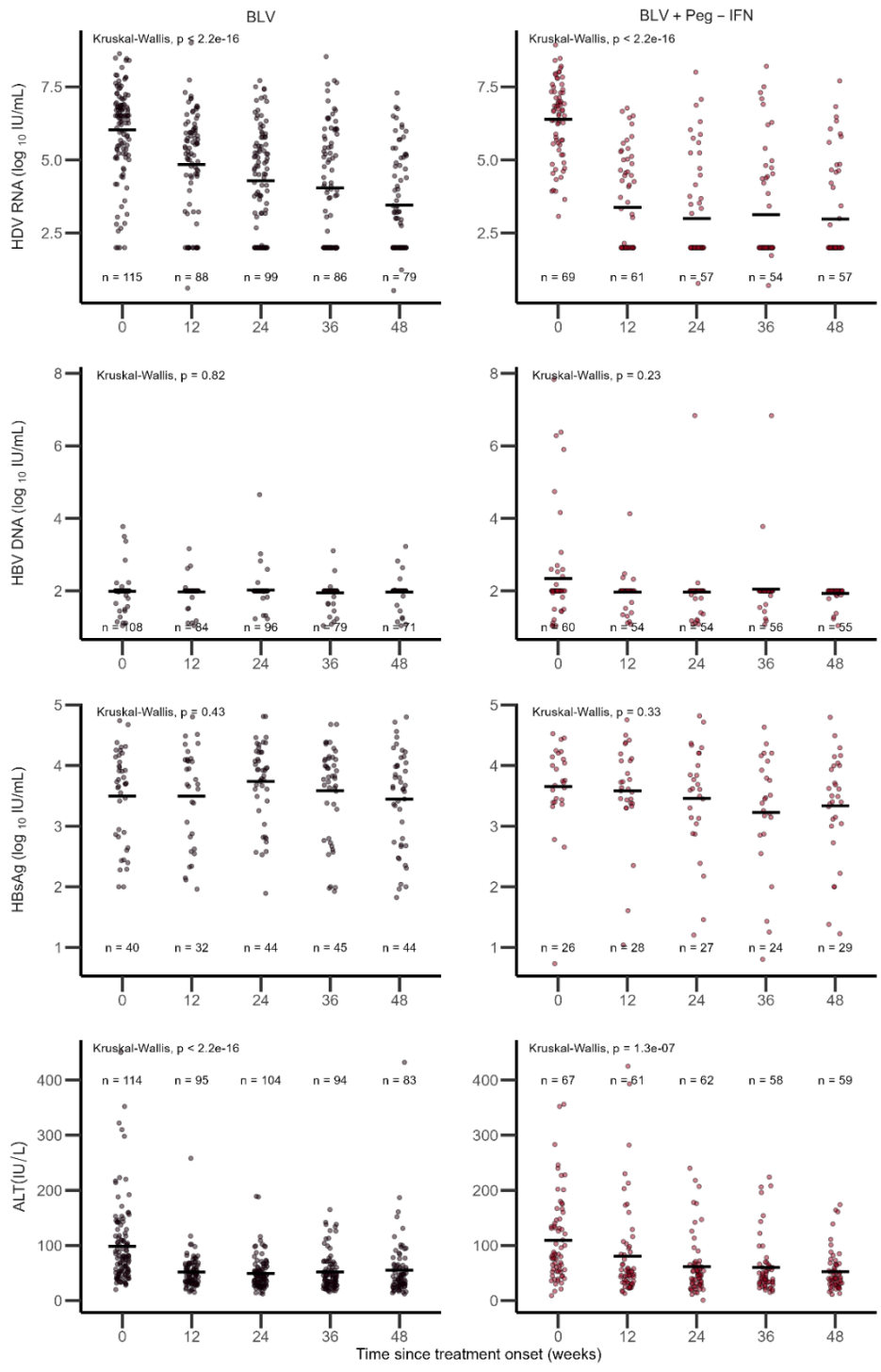


Figure S5: Virologic and biochemical decline kinetics in each group. The plain line represents the median,n represent the number of observations

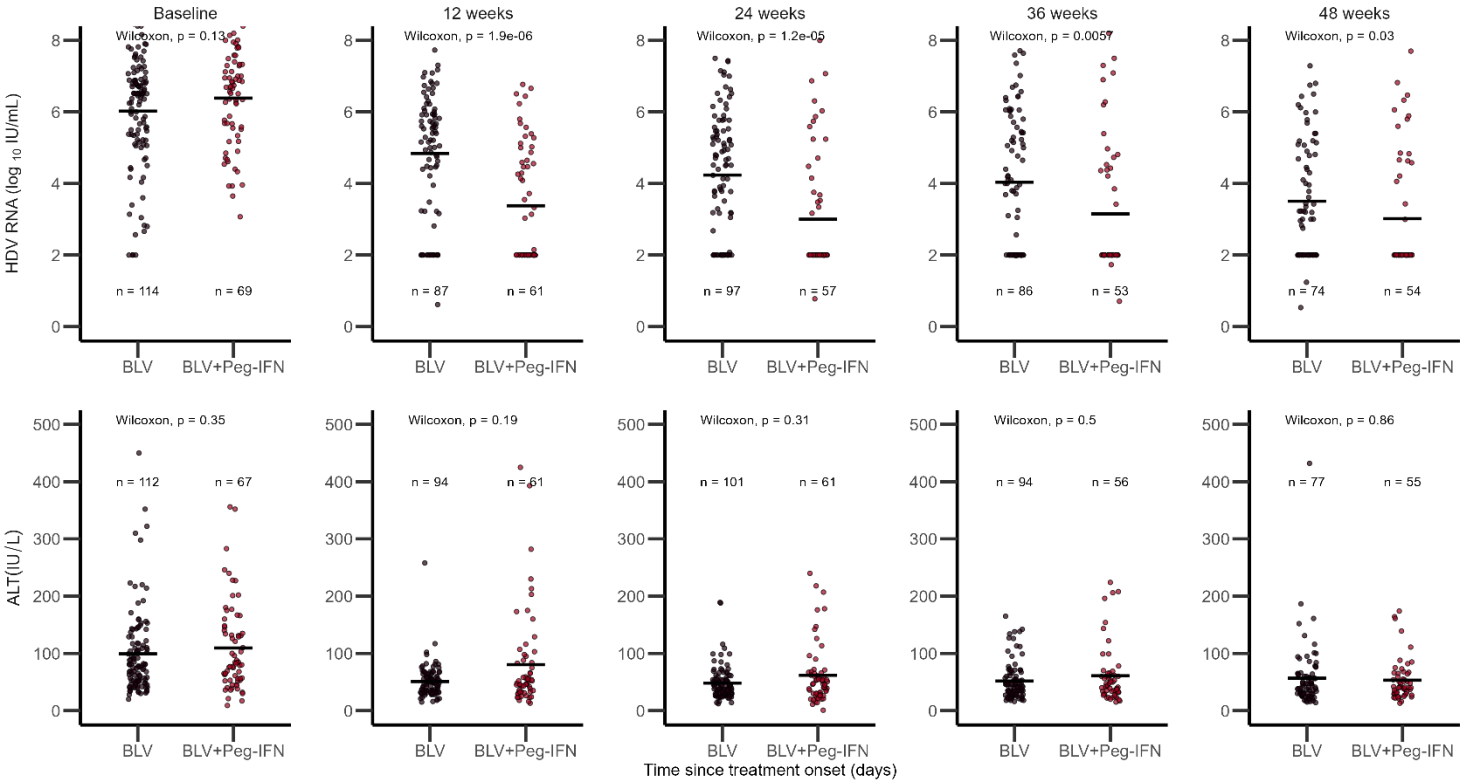


Figure S6: Distribution of the observed HDV RNA and ALT levels across the study in groups Bulevirtide (BLV) and bulevirite+Peg-IFN (BLV+Peg-IFN). The plain line represents the median concentration, n represent the number of observations.

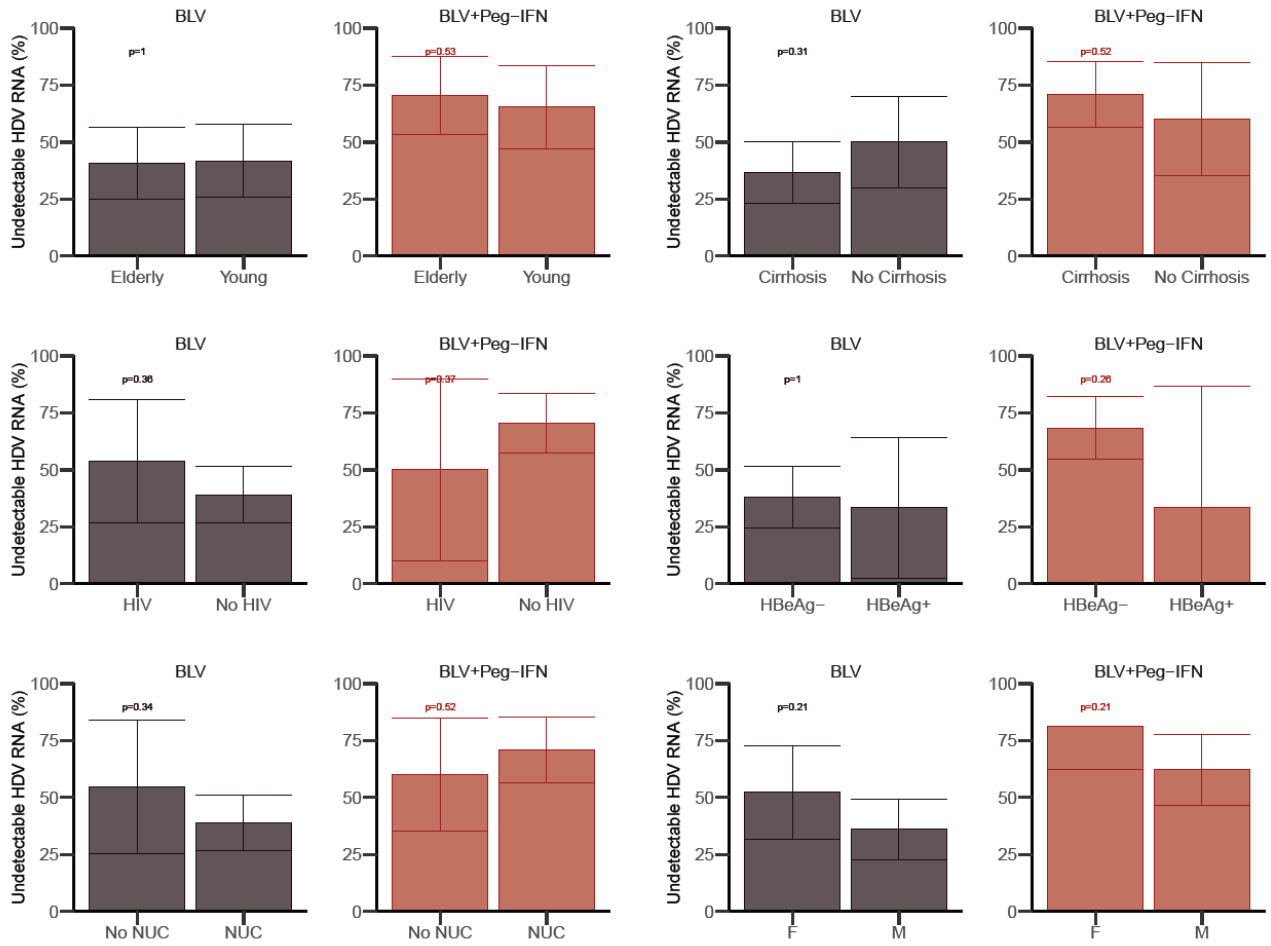


Figure S7 : Percentage of undetectable HDV RNA in each group according to baseline covariates. The errorbars correspond to the 95% confidence interval of a binomial law.

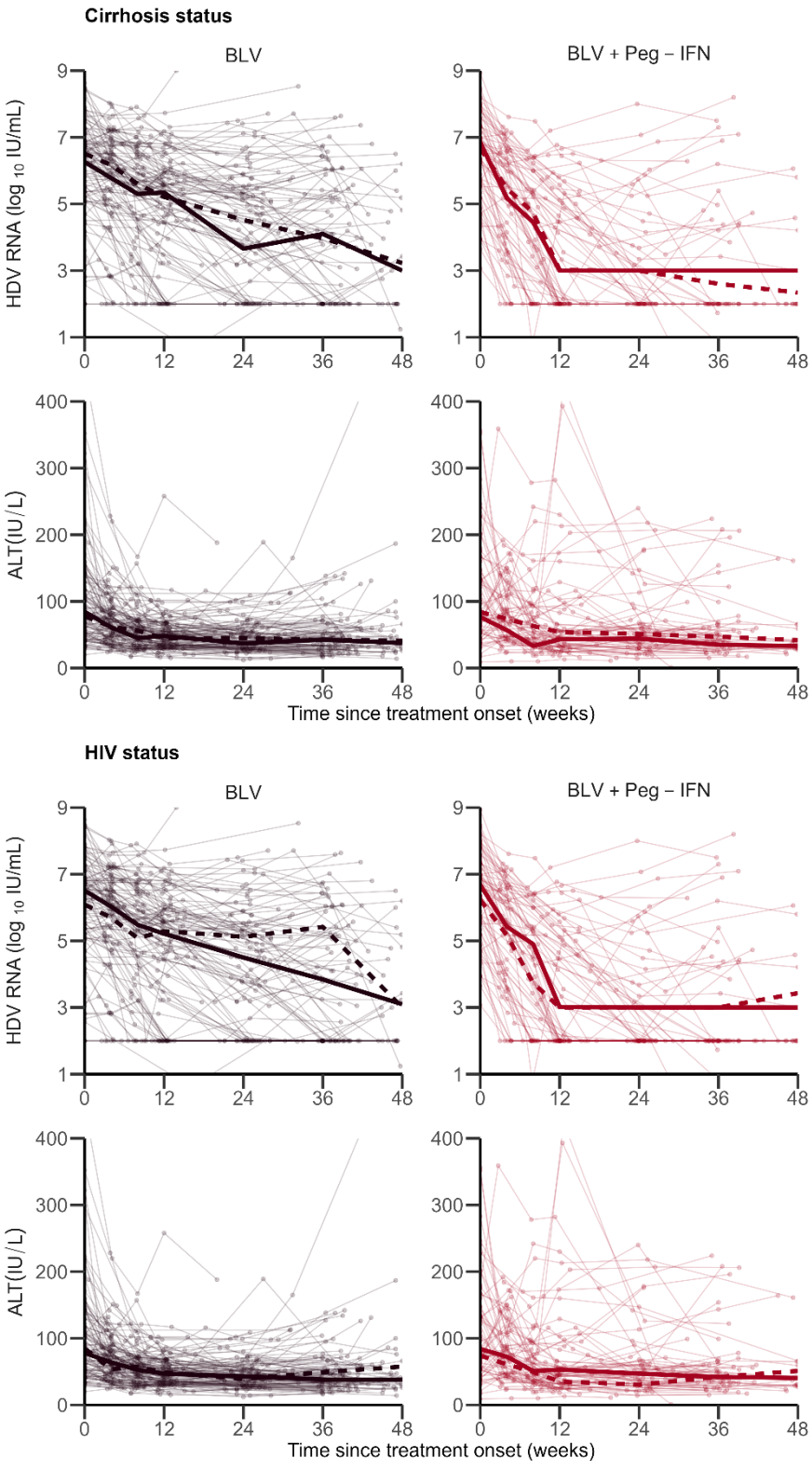


Figure S8 : HDV RNA and ALT kinetics observed in each group. The large line correspond to the median observed in top : cirrhotic patients (plain line) versus non-cirrhotic patients (dashed line) ; bottom : HIV patients (plain line) versus non-HIV patients (dashed line).

4. Calibration curves

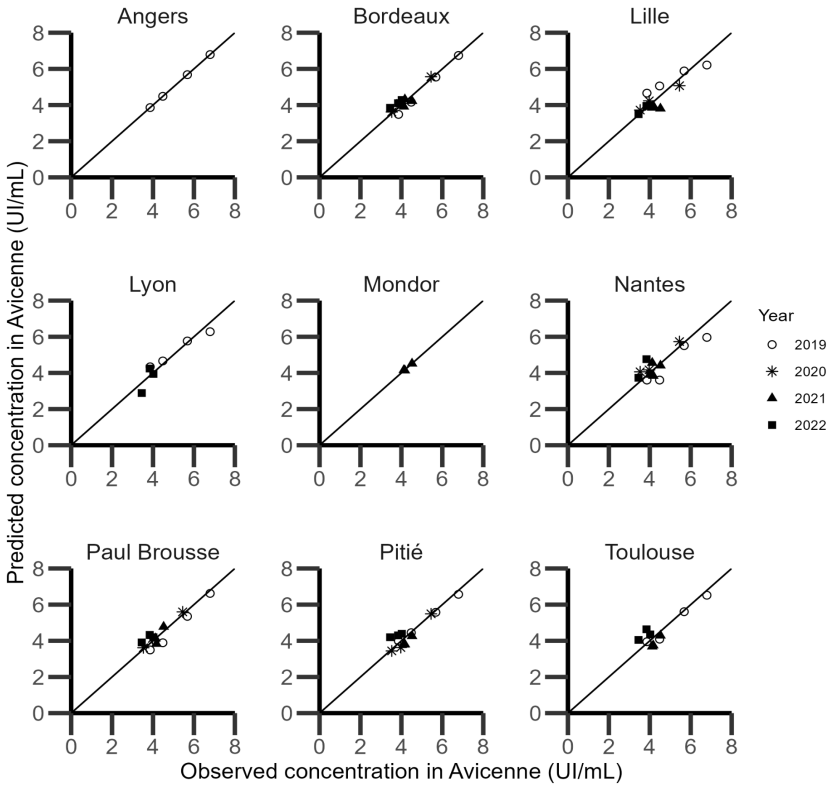


Figure S9: Measurements of the samples used to derive the linear regression, with their value with "home-made" techniques and the reference technique.

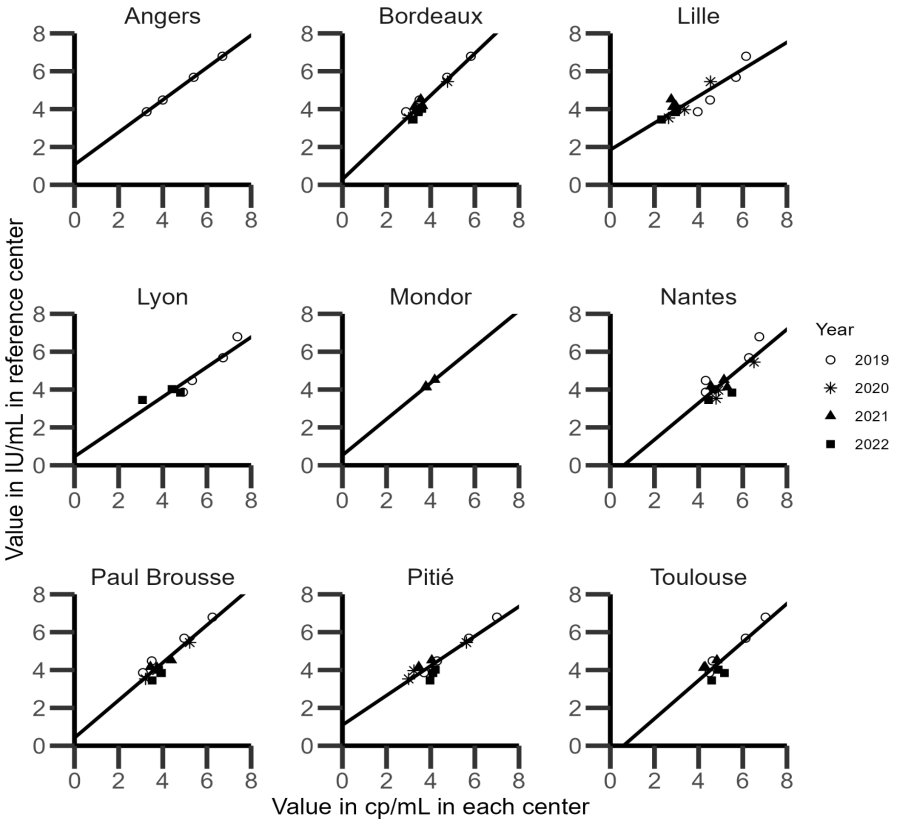


Figure S10: Linear regression curves allowing to derive concentrations from the reference technique.

5. Survival analysis : Probability of stopping treatment

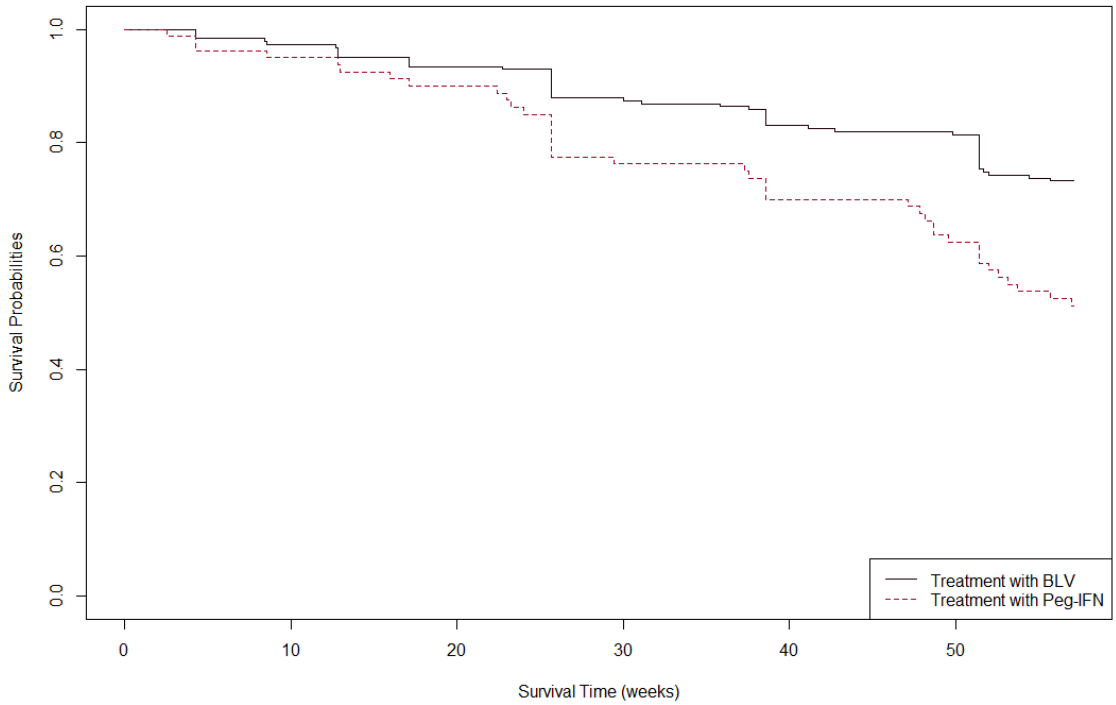


Figure S11: Kaplan-Meyer curves on the survival probability of treatment cessation.

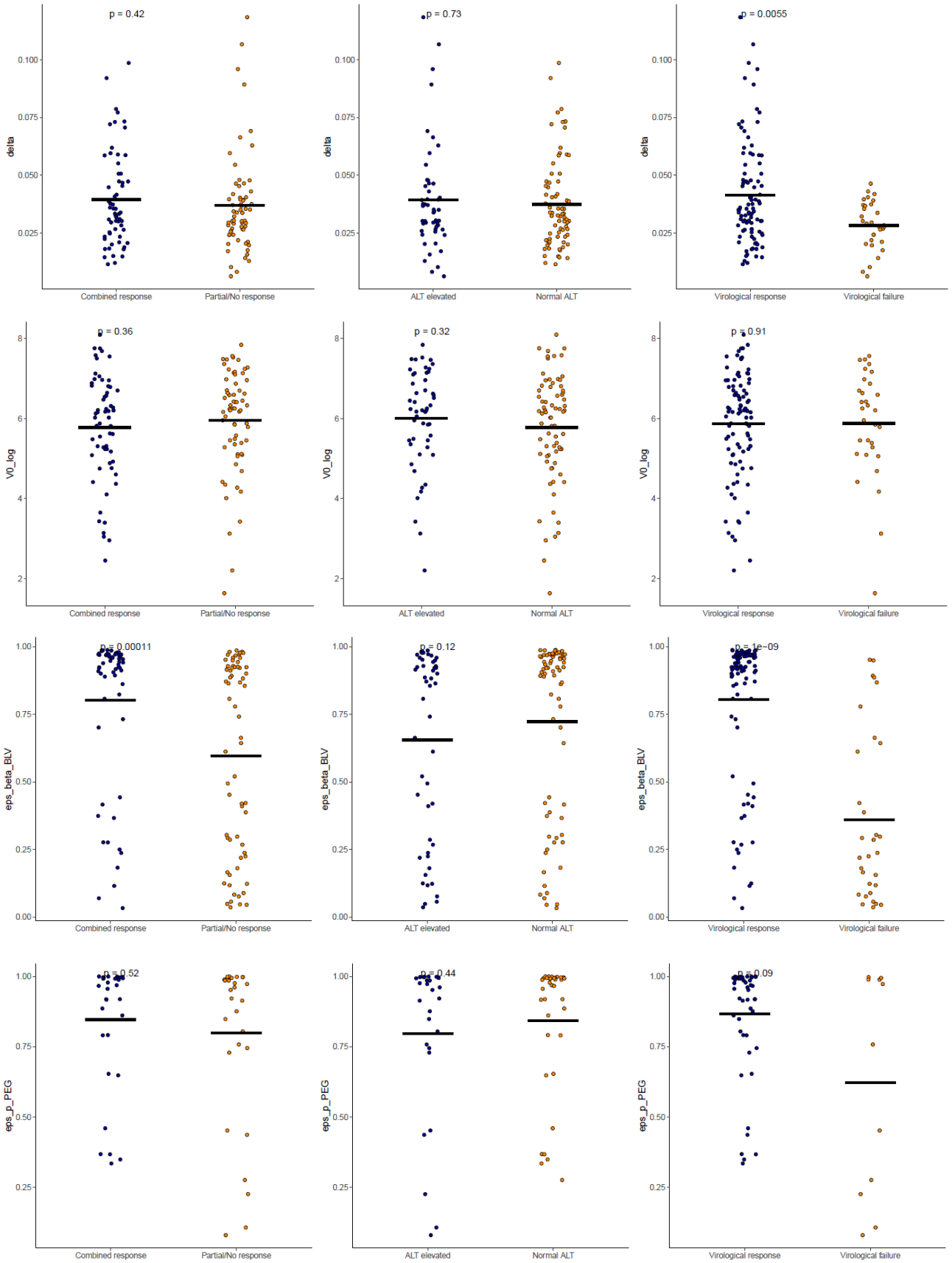
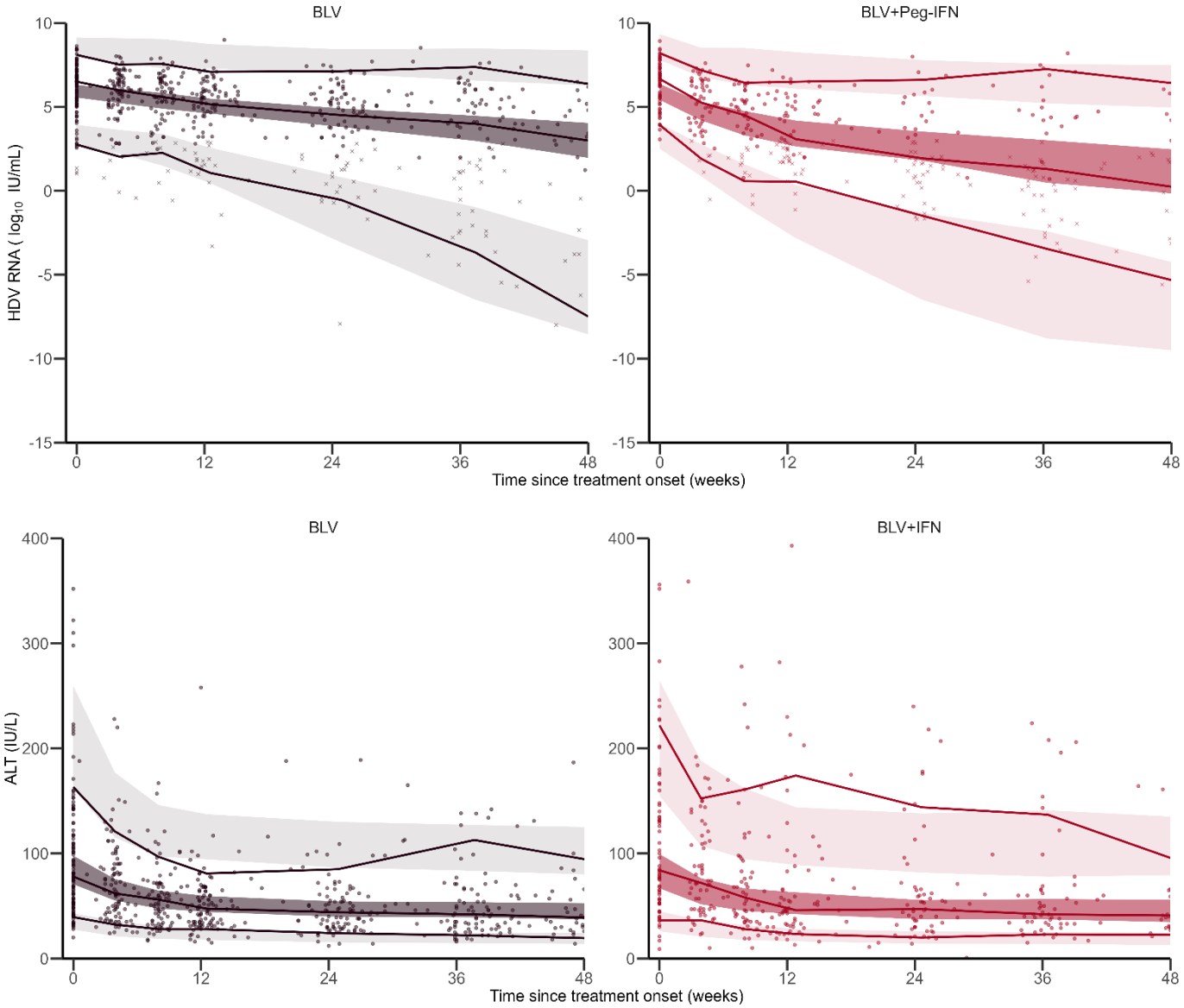


Figure S12 : Distribution of parameters according to the virological, biochemical or combined response of the patients.

6. Model evaluation



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

Figure S13: Visual predictive checks (VPCs) stratified on arms Bulevirtide monotherapy (BLV) or in combination with Peg-IFN. Dots represent observed values, plain lines represent the 5th, 50th and 95th empirical percentiles on observed data. Shaded areas represent the 95% prediction interval around the corresponding percentiles.

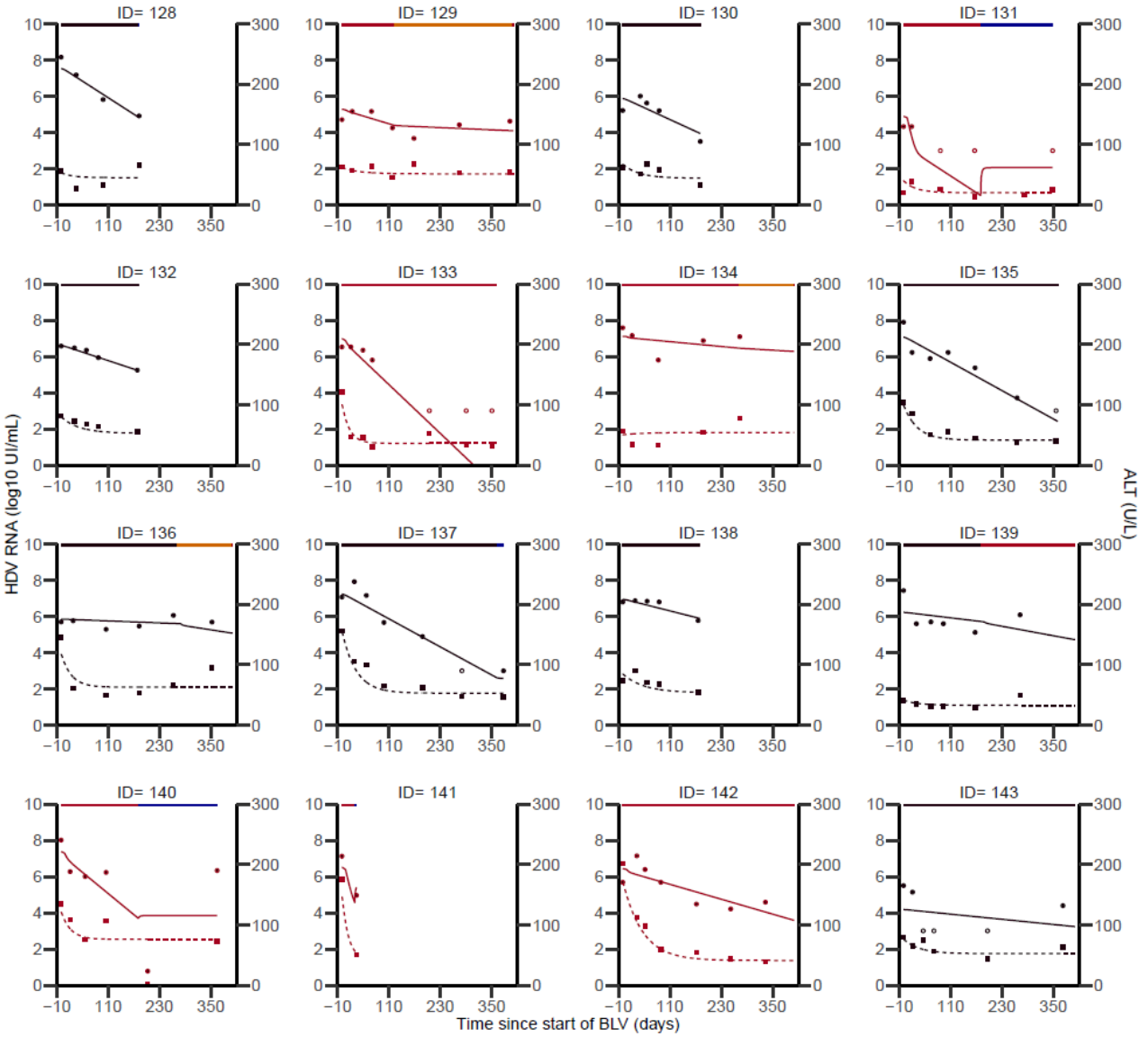


Figure S14: Individual predictions of HDV RNA (plain line) and ALT (dotted lines) in patients in the group Bulevirtide (black lines) or bulevirtide+Peg-IFN (red lines). The top plain line indicates the treatment received at each time (Red, Black, orange and blue for BLV+Peg-IFN, BLV only, Peg-IFN only and off-treatment, respectively).

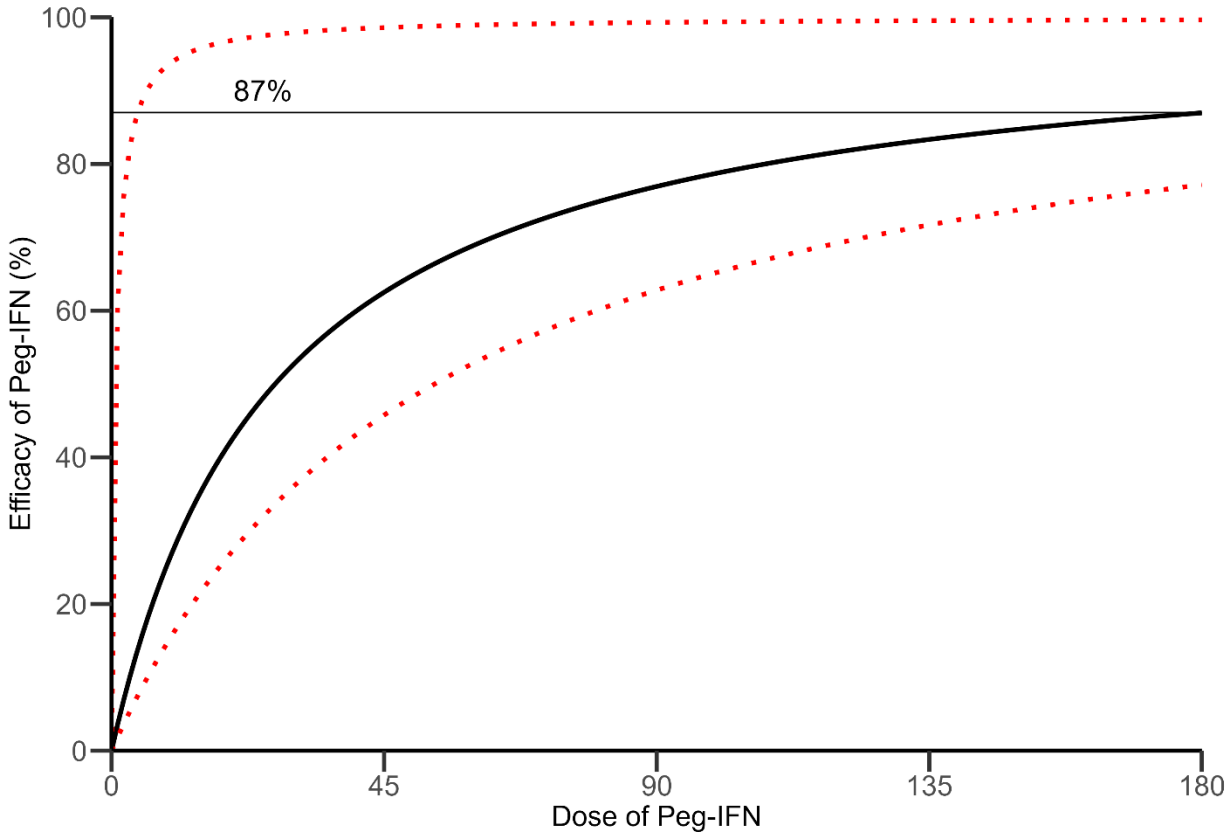


Figure S15: Relationship between the dose of Peg-IFN and the efficacy on blocking viral production in the sensitivity analysis taking into account the dose.

Tables

1. Limit of quantification

Table S1 : Summary of the number of observation associated with the different limit of quantifications (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

2. Estimation of treatment effect

Table S2 : Results of the model building of drug inclusion

		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

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

506

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)
$\mathbf{ED90}^{PEG}$	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)	

508

509

510

511

512

513

514

515

4. Model prediction : comparison to MY204 study

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