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Using Pharmacokinetic and Viral Kinetic Modeling To Estimate the Antiviral Effectiveness of Telaprevir, Boceprevir, and Pegylated Interferon during Triple Therapy in Treatment-Experienced Hepatitis C Virus-Infected Cirrhotic Patients.

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1 **Using pharmacokinetic and viral kinetic modeling to estimate the antiviral effectiveness**
2 **of telaprevir, boceprevir and Peg-IFN during triple therapy in treatment-experienced**
3 **HCV infected cirrhotic patients (ANRS CO20-CUPIC)**

4

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23

24 **Running Head:** Effectiveness of triple therapy in cirrhotic patients

25

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27

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31

32 **Abstract**

33 **Background** Triple therapy combining a protease inhibitor (PI) telaprevir or boceprevir,
34 pegylated-interferon (Peg-IFN) and ribavirin (RBV) have dramatically increased the chance
35 to eradicate hepatitis C virus (HCV). However the efficacy of this treatment remains
36 suboptimal in cirrhotic experienced-patients. Here we aimed to better understand the origin of
37 this impaired response by estimating the antiviral effectiveness of each drug.

38 **Methods** Fifteen genotype 1-patients with compensated cirrhosis, non-responders to a prior
39 Peg-IFN/RBV therapy were enrolled in a non-randomized study. HCV-RNA and drug
40 concentrations of PIs, Peg-IFN and RBV were frequently assessed in the first 12 weeks of
41 treatment and were analyzed using a pharmacokinetics/viral kinetics model.

42 **Results** Both PIs achieved similar level of molar concentrations ($P=0.5$), but there was a
43 significant difference of EC_{50} ($P=0.008$), leading to a larger antiviral effectiveness than
44 boceprevir in blocking viral production (99.8% vs 99.0%, respectively, $P=0.002$). In all
45 patients the antiviral effectiveness of Peg-IFN was modest (43.4%) and there was no
46 significant contribution of RBV exposure on the total antiviral effectiveness. The second
47 phase of viral decline, which is attributed to the loss rate of infected cells, was slow (0.19 day^{-1})
48 and was higher in patients that subsequently eradicated HCV ($P=0.03$).

49 **Conclusion** Both PIs achieved a high level of antiviral effectiveness. However the suboptimal
50 antiviral effectiveness of Peg-IFN/RBV and the low loss of infected cells suggest that longer
51 treatment duration might be needed in cirrhotic treatment experienced-patients and that future
52 IFN-free regimen may be particularly beneficial to these patients.

53

54 **Keywords:** Hepatitis C virus; Non-linear mixed effect models; Early viral kinetics; Protease
55 inhibitor; Pegylated-interferon; Ribavirin; Mathematical modeling; Pharmacokinetic

56

57 **Introduction**

58 Chronic infection with hepatitis C virus (HCV) affects approximately 160 million people
59 worldwide (1) and is the leading cause of cirrhosis, liver cancer and liver transplantation (2).
60 The goal of treatment is to achieve a sustained virological response (SVR), marker of viral
61 eradication, assessed by the absence of detectable HCV RNA six months after treatment
62 discontinuation. The approval in 2011 of two protease inhibitors (PI), telaprevir and
63 boceprevir, in combination with pegylated-interferon-alpha and ribavirin (Peg-IFN/RBV) (3),
64 has marked an important milestone with SVR rates higher than 70% in HCV genotype 1
65 infected patients (4, 5). Recently two new triple therapy involving sofosbuvir, a nucleoside
66 polymerase inhibitor, and simeprevir, a new protease inhibitor, have been approved by the
67 European and American regulatory agencies, showing in clinical trials even higher SVR rates
68 of 90% (6). However the cost of these new treatments, about twice as much as telaprevir or
69 boceprevir-based therapy (7), will make them out of reach for many countries. Therefore
70 triple therapy with Peg-IFN, RBV and telaprevir/boceprevir will continue to be vastly used in
71 the next years and will remain the only therapeutic option for many patients.

72 Although these results suggest that a functional cure might be obtained in a large majority of
73 patients, one should keep in mind that issues remain. In particular the proportion of patients
74 with advanced liver disease and cirrhosis and/or who had failed a previous treatment with
75 Peg-IFN/RBV is under represented in the patient population in clinical trials (8–11). The
76 evaluation of the triple therapy in this population was precisely the goal of the ANRS-CO20-
77 CUPIC cohort (Compassionate Use of Protease Inhibitors in viral C Cirrhosis;
78 ClinicalTrials.gov number: NCT01514890) (12), where 511 genotype 1 treatment-
79 experienced cirrhotic patients were included. In this study the SVR rates 12 weeks after
80 treatment discontinuation (SVR12) were equal to 52% and 43% in telaprevir and boceprevir
81 treated patients, respectively (13). The origin of this impaired response might encompass a

82 variety of factors, in particular impaired drug pharmacokinetics (PK) or limited sensitivity to
83 PI agents and/or Peg-IFN/RBV in this particular population.

84 One way to evaluate treatment antiviral effectiveness and to optimize therapy is to use PK-
85 viral kinetic (VK) models that provide a useful tool to quantitatively describe the relationship
86 between drug exposure and viral response (reviewed in (14)). However no such analysis has
87 been published with boceprevir and results published for telaprevir were mostly based on
88 treatment naive and/or non-cirrhotic patients (15–17).

89 Here, we aimed to get new insights into the determinants of the response to triple therapy by
90 analyzing in details, within a subset of 15 patients enrolled in the ANRS-CO20-CUPIC study,
91 the relationship between drug concentrations and early virological response. We used the
92 techniques of PK-VK modeling in order to tease out the relative antiviral effectiveness of
93 each of the agents involved in the triple therapy (*i.e.*, boceprevir or telaprevir, Peg-IFN and
94 RBV) and to investigate for a possible association with long term virological response.

95

96 **Materials and methods**

97 **Patients and data**

98 MODCUPIC is a substudy of the French multicentre prospective ANRS-CO20-CUPIC
99 cohort. In four centres, from September 2011 to September 2012, patients chronically
100 monoinfected with HCV genotype 1, compensated cirrhosis (Child-Pugh class A), non-
101 responders to a prior IFN-based therapy and who started triple therapy were recruited. The
102 diagnosis of cirrhosis was made by liver biopsy or non-invasive tests, Fibrotest® or
103 Fibroscan® or Fibrometer® or Hepascore® at the discretion of the investigator, according to
104 the French recommendations (18). The choice between TVR- or BOC-based therapies was at
105 the investigator's discretion without randomization. TVR-based therapy included 12 weeks of
106 telaprevir (750 mg/8 hours) in combination with Peg-IFN- α 2a (180 μ g/week) and RBV (1,000
107 or 1,200 mg/day, depending on body weight) then 36 weeks of Peg-IFN- α 2a/RBV (named
108 group telaprevir in the following). BOC-based therapy included 4 weeks (lead-in phase) of
109 Peg-IFN- α 2b (1.5 μ g/kg/week) or Peg-IFN- α 2a (180 μ g/week) and RBV (800 or 1,400
110 mg/day, depending on body weight) then 44 weeks of Peg-IFN- α 2b/RBV and boceprevir (800
111 mg/8 hours) (named group boceprevir in the following). Patients were followed up to six
112 months after treatment discontinuation to assess SVR.

113 Written informed consent was obtained before enrolment. The protocol was conducted in
114 accordance with the Declaration of Helsinki and was approved by the "Ile-de-France IX
115 Ethics Committee" (Créteil, France).

116

117 **Bioanalytical methods**

118 HCV RNA and drug concentrations were measured post PIs initiation at hours 0, 8, days 1, 2,
119 3 and weeks 1, 2, 3, 4, 8 and 12. Patients treated with boceprevir had two additional VL and
120 concentrations measurements during the lead-in phase. Blood samples were collected early in

121 the morning before the first daily dose of PIs and RBV and therefore only trough pre-dose
122 drug concentrations were collected. All samples were collected on SST (serum) vacutainers,
123 kept at 4°C until centrifuged at 3,000 RPM for 10 minutes in a 4°C centrifuge, within 1 hour
124 after collection, aliquoted and kept at -80°C until analysis.

125 PIs concentrations in serum were determined using ultra-performance liquid chromatography
126 coupled with tandem mass spectrometry with a lower limit of quantification (LOQ) of 5 ng/ml
127 and 10 ng/ml for boceprevir and telaprevir, respectively (19). PI concentrations were
128 converted to $\mu\text{mol/l}$ for analysis using molar masses of 519.68 g/mol and 679.85 g/mol for
129 boceprevir and telaprevir, respectively. RBV concentrations in serum were determined using
130 ultra-performance liquid chromatography coupled with UV detection with a LOQ of 100
131 ng/ml (20). Peg-IFN- α 2a and - α 2b in serum were determined with a bioassay which was
132 chosen because the objective was to quantify the antiviral activity of Peg-IFN- α and not only
133 the concentration. Immunoassay measures the physical quantity of material but does not
134 differentiate between active and inactive molecules while bioassay for IFN- α is based on the
135 protection of cultured cells against the cytopathic effect of a challenge virus and also was
136 suitable for assaying both Peg-IFN- α -2a and Peg-IFN- α -2b. The reference solutions
137 contained 2.8–180 ng/ml of Peg-IFN- α 2a (Roche Diagnostics, Germany) (21).

138 HCV-RNA levels were measured with a real-time PCR-based assay, Cobas®
139 Ampliprep/Cobas TaqMan® assay (Roche Diagnostics, Germany), with a lower limit of
140 detection (LOD) of 15 IU/ml. DNA samples were genotyped for the IL28B rs12979860
141 polymorphism (AmpliTaq gold® DNA polymerase and BigDye® terminator cycle
142 sequencing kit, Applied Biosystems, UK).

143

144 **Drug pharmacokinetic modeling**

145 All drug concentrations were fitted separately in telaprevir and boceprevir treatment groups.
 146 For both Peg-IFN and RBV, the trough serum concentrations, noted $C^{Peg-IFN}(t)$ and $C^{RBV}(t)$,
 147 respectively were fitted using an exponential model to reflect the progressive increase in
 148 trough drug concentrations over time:

$$149 \quad C^{Peg-IFN}(t) = C_{ss}^{Peg-IFN} \times (1 - e^{-kt}) \quad \text{Eq. (1)}$$

$$150 \quad C^{RBV}(t) = C_{ss}^{RBV} \times (1 - e^{-kt}) \quad \text{Eq. (2)}$$

151 where C_{ss} is the trough concentration at steady state and k the rate constant of elimination
 152 which reflects the progressive increase in $C(t)$ over time.

153 For both PI drugs, consistent with the fact that they have a short elimination half-life (22), no
 154 significant increase of trough concentrations over time was observed. Therefore
 155 concentrations for both telaprevir and boceprevir were fitted using a constant model, where
 156 C_{ss} is the trough concentration:

$$157 \quad C^{PI}(t) = C_{ss}^{PI} \quad \text{Eq. (3)}$$

158

159 **Viral kinetic modeling**

160 The following model of HCV viral kinetics (VK) was used to fit the changes in HCV RNA
 161 (23):

$$162 \quad \frac{dI}{dt} = bVT - \delta I$$

$$163 \quad \frac{dV}{dt} = p(1 - \varepsilon(t))I - cV \quad \text{Eq. (4)}$$

164 where T represent the target cells that can be infected by virus, V , with rate b . Infected cells, I ,
 165 are lost with rate δ and produce p virions per day, which are cleared from serum with rate c .
 166 The target cell level is assumed constant throughout the study period (12 weeks) and remains
 167 at its pre-treatment value $T_0 = c\delta/p\beta$. Treatment is assumed to reduce the average rate of viral
 168 production per cell from p to $p(1-\varepsilon)$, where ε represents the drug antiviral effectivenesses, *i.e.*,
 169 $\varepsilon = 0.99$ implying the drug is 99% effective in blocking viral production. This model predicts

170 that VL will fall in a biphasic manner, with a rapid first phase lasting for a couple of days that
 171 reduce the VL with a magnitude equal to $\log_{10}(1-\varepsilon)$, followed by a second slower but
 172 persistent second phase of viral decline with rate $\varepsilon\delta$. Therefore a difference between $\varepsilon =$
 173 99.9% and $\varepsilon = 99.0\%$ corresponds to a 10-fold difference in the viral production under
 174 treatment and will lead to 1-log difference between the two curves of viral decline (24). We
 175 fixed p and b to 100 IU/ml/cell/day and 10^{-7} (IU/ml)⁻¹/day, respectively, without loss of
 176 generality (25).

177 The effectiveness of each drug in blocking viral production was described by an E_{\max} model
 178 assuming a maximum inhibition of 100%:

$$\begin{aligned}
 179 \quad \varepsilon^{PI}(t) &= \frac{C^{PI}(t)}{C^{PI}(t) + EC_{50}^{PI}} \\
 180 \quad \varepsilon^{Peg-IFN}(t) &= \frac{C^{Peg-IFN}(t)}{C^{Peg-IFN}(t) + EC_{50}^{Peg-IFN}} \quad \text{Eq. (5)}
 \end{aligned}$$

181 where EC_{50}^{PI} (respectively $EC_{50}^{Peg-IFN}$) is the PI (resp. Peg-IFN) concentration at which the PI
 182 (resp. Peg-IFN) is 50% effective, and $C^{PI}(t)$ (resp. $C^{Peg-IFN}(t)$) are the individual predictions
 183 (see below) given by the PK models (Eq. 1 and 3).

184 The combined effect of PIs and Peg-IFN was modeled using a Bliss independent action model
 185 (26) and the total efficacy $\varepsilon(t)$ was given by:

$$186 \quad (1 - \varepsilon(t)) = (1 - \varepsilon^{PI}(t))(1 - \varepsilon^{Peg-IFN}(t)) \quad \text{Eq. (6)}$$

187 Since the effect of RBV on the early virological response is expected to be modest (27–29)
 188 we did not incorporate the effect of RBV into the reference model (Eq. 4-6). In a second step
 189 we tested whether the effectiveness of RBV, also modeled using an E_{\max} model could enhance
 190 the effect in blocking viral production or reduce viral infectivity, as suggested previously (30).

191

192 **Data analysis and parameter estimation**

193 The pharmacokinetics/viral kinetics (PK-VK) model given by Eq. 4-6 can be used only to
194 characterize the viral kinetics of drug sensitive virus and therefore cannot fit viral rebounds
195 due to the emergence of drug-resistant virus. Therefore only HCV RNA data until virologic
196 rebounds (with no indication of lack of compliance) were used to estimate the viral kinetic
197 parameters.

198 Parameters V_0 , c , δ , EC_{50}^{PI} and $EC_{50}^{Peg-IFN}$ were estimated using non-linear mixed-effect
199 models (NLMEM). In this approach, each individual parameter θ_i is comprised of a fixed part
200 θ , which represents the mean value of the parameter in the population (fixed effects), and a
201 random part η_i chosen from a Gaussian distribution with mean 0 and standard deviation ω_i
202 that accounts for the inter-individual variability. Therefore, for all parameters $\theta_i = \theta e^{\eta_i}$
203 where $\eta_i \sim N(0, \omega^2)$. Both PK data and $\text{Log}_{10}(\text{HCV RNA})$ were best described using an
204 additive residual error with constant variance.

205 Model parameters were estimated using the Stochastic Approximation Expectation
206 Minimization (SAEM) algorithm in MONOLIX v4.2 (available at <http://www.lixoft.eu>). Of
207 note this approach is based on maximum likelihood estimation which take into account the
208 information brought by data under the LOD as left-censored data (31, 32).

209 Model selection was done using the Bayesian information criteria (BIC), a fitting criterion
210 derived for each model from the computation of likelihood that takes into account the number
211 of estimated parameters used (the lower the better (33)). Model evaluation was performed
212 using goodness-of-fit plots, as well as the individual weighted residuals (IWRES) and the
213 normalized prediction distribution errors (NPDE) over time.

214

215 **Difference in PK-VK model parameters between telaprevir and boceprevir treatment**
216 **group**

217 A Wald test on the PK-VK model parameters (c, δ, EC_{50}^{PI}) was used to assess the difference
 218 in population parameters between the two groups. Because we previously showed that this
 219 approach could lead to an inflation of the type I error in case of small sample size ($N < 20$ per
 220 group) (34), a permutation test was performed to confirm statistical significance when the
 221 Wald test was significant at the level of 5%. In brief, 1,000 datasets were simulated by
 222 randomly allocating patients to telaprevir or boceprevir group, maintaining a similar
 223 proportion of patients allocated to each groups than in the original dataset. Then the P-value
 224 of the Wald test was calculated for each simulated data set. Finally the corrected P-value of
 225 the permutation test is equal to the proportion of simulated datasets having a P-value lower
 226 than the one found one the original dataset.

227 Because the genetic barrier to resistance of PI (*i.e.*, the number of change in amino acids
 228 needed to generate mutants with high level of resistance) depends of HCV subgenotype and
 229 therefore lead to different SVR rate, we also estimated the effect of HCV subgenotype (1a vs
 230 non-1a) on viral kinetic parameters. IL28B polymorphism, which is also associated with
 231 response to IFN-based therapy, was not investigated because all these patients had failed to a
 232 previous bitherapy.

233

234 **Prediction and comparison of individual parameters**

235 Individual Empirical Bayesian Estimates (EBE) parameters for both PK and VK were
 236 obtained by computing for each patient the Maximum A Posteriori (MAP) estimate. The
 237 individual antiviral effectiveness at steady state, ϵ_{ss} , of each agent was defined by:

238

$$\epsilon_{ss}^{PI} = \frac{C_{ss}^{PI}}{C_{ss}^{PI} + EC_{50}^{PI}}$$

239

$$\epsilon_{ss}^{Peg-IFN} = \frac{C_{ss}^{Peg-IFN}}{C_{ss}^{Peg-IFN} + EC_{50}^{Peg-IFN}} \quad \text{Eq. (7)}$$

240 Non-parametric two-sided tests (Wilcoxon test) were used to compare i) individual EBE PK
241 parameters between patients who received telaprevir vs boceprevir and between patients who
242 received Peg-IFN- α 2a vs - α 2b, and ii) individual EBE PK parameters between SVR and non-
243 SVR patients. Because all patients were non-responder to Peg-IFN, the effect of IL28B
244 genotype on PK and VK parameters was not tested.
245

246 Results

247 Fifteen HCV genotype 1 patients were included 9 receiving telaprevir and 6 receiving
248 boceprevir. Twelve (80%) were men, with a median [min; max] age of 55 [44; 64] years.
249 Seven (47%) patients were infected with subgenotype 1a, 2 (22%) in telaprevir group and 5
250 (83%) in boceprevir group. Prior treatment responses were partial response, null response,
251 relapse and early discontinuation for adverse events in 2, 5, 6 and 2 patients, respectively.
252 Only two patients had the most favorable IL28B CC genotype (35). Main characteristics of
253 the patients are presented in Table 1.

254 Two patients had a viral breakthrough (at weeks 3 and 8). Eleven patients received Peg-IFN-
255 α 2a (8 in telaprevir group and 3 in boceprevir group), 3 patients Peg-IFN- α 2b (all in
256 boceprevir group) and one patient in telaprevir group did not receive any injection of Peg-IFN
257 (and this patient had a viral breakthrough at week 3).

258 Fig. 1 shows the observed drug concentrations versus time and Table 2 gives the estimated
259 steady state trough concentrations, C_{ss} , for all drugs. There was no significant difference in
260 the molar medians steady state concentrations of telaprevir and boceprevir ($C_{ss}^{telaprevir} = 3.77$
261 [2.68; 5.98] $\mu\text{mol/l}$ *i.e.* 2,563.0 ng/ml [1,822.0; 4,065.5] and $C_{ss}^{boceprevir} = 3.92$ [3.22; 7.64]
262 $\mu\text{mol/l}$ *i.e.* 2037.1 ng/ml [1,673.4; 3,970.4], $P=0.5$). There was no significant difference in the
263 median steady state concentrations of Peg-IFN- α 2a and - α 2b ($C_{ss}^{Peg-IFN-2a} = 89.6$ [52.8; 110.4]
264 ng/ml and $C_{ss}^{Peg-IFN-2b} = 55.4$ [55.3; 57.9] ng/ml, $P=0.2$). The concentrations of RBV increased
265 over time in all patients and could be well captured by our model (Eq. 2) with a median k
266 equal to 0.10 day^{-1} , corresponding to a half-life of increase of about 7 days. At equilibrium
267 medians C_{ss}^{RBV} were equal to 2,860 [2,428; 3,874] ng/ml.

268 After the PK parameters were estimated, the predicted individual PK time courses were
269 plugged into the PK-VK model (see methods). Baseline VL was higher in the telaprevir group
270 than in the boceprevir group, thus a treatment group effect was added on baseline VL

271 ($V_0^{telaprevir} = 6.43 \log_{10}$ IU/ml vs $V_0^{boceprevir} = 5.52 \log_{10}$ IU/ml, $P=0.0001$). A greater proportion
272 of patients that received boceprevir were genotype 1a relative to those that received telaprevir
273 ($P=0.04$). Subgenotype is an important predictor of the response to treatment, in particular
274 with telaprevir with a lower genetic barrier to resistance with genotype 1a than 1b (only one
275 nucleotide change in genotype 1a viral genomes is required to generate mutations V36M and
276 R155K/T, vs two in genotype 1b) (36). This may explain why genotype 1a patients were
277 preferentially treated with boceprevir. We did not find any significant effect of subgenotype
278 on any of the parameters.

279 The model could well describe the kinetics of HCV decline observed both during the lead-in
280 phase (in the boceprevir group) and after the initiation of the PIs (in both groups, see Fig. 2).
281 There was no evidence of model misspecification as showed by the goodness-of-fit plot (Fig.
282 3) and all parameters could be estimated with a good precision (Table 3).

283 The model predicted a mean $EC_{50}^{Peg-IFN}$ equal to 106 ng/ml, leading to a low antiviral
284 effectiveness at steady state of Peg-IFN at steady state of 43.4% [0.0; 52.7], consistent with
285 the modest 0.67 \log_{10} IU/ml drop observed during the four weeks lead-in phase in patients
286 treated with boceprevir (Fig. 2).

287 After PI initiation, VL declines in a biphasic manner in all patients, where a rapid first phase
288 was followed by a second slower phase. The rapid first phase was attributed to a clearance
289 rate of virus, c , equal to 3.98 day^{-1} and to a high level of antiviral effectivenesses for both PIs.
290 The intrinsic potency of the two molecules, as measured by the EC_{50}^{PI} , was significantly
291 higher for telaprevir than boceprevir ($EC_{50}^{telaprevir} = 0.009 \mu\text{mol/l}$ vs $EC_{50}^{boceprevir} = 0.04$
292 $\mu\text{mol/l}$, $P=0.008$). Importantly the statistical significance of this difference was obtained after
293 taking into account the small sample size (see methods) and adjusted on baseline VL. Since
294 telaprevir had a lower EC_{50} than boceprevir and that both drugs achieved similar levels of
295 molar concentrations the model predicted that the median individual antiviral effectiveness of

296 PI agent in blocking viral production was significantly higher in patients that received
297 telaprevir than in those who received boceprevir ($\varepsilon_{ss}^{telaprevir} = 99.8\% [99.3; 99.9]$ and $\varepsilon_{ss}^{boceprevir}$
298 $= 99.0\% [98.0; 99.6]$, $P=0.002$). Interestingly this model could well capture the relationship
299 between the serum exposure and its antiviral effectiveness, demonstrating that the variability
300 in drug exposure needs to be taken into account to understand the between-subject variability
301 in PIs antiviral effectiveness (Fig. 4A). Lastly because the effectiveness of both PIs were
302 much larger than that of Peg-IFN (Fig. 4B), the total antiviral effectiveness obtained by the
303 combination of PI and Peg-IFN was largely similar to the one obtained with the PIs only.

304 After the VL was rapidly reduced as a result of the strong antiviral effectiveness of both PIs,
305 the model predicted that a second slower phase of viral decline ensued, driven by the loss rate
306 of infected cells, δ . We estimated δ to be equal to 0.18 day^{-1} , corresponding to a half-life of
307 infected cells of 3.9 days, with no significant differences between patients receiving telaprevir
308 and boceprevir ($P=0.5$).

309 Next we investigated the relationship between the PK-VK parameters and SVR. Among the 7
310 patients (47%) who achieved SVR, 5 received telaprevir and 2 received boceprevir (56% vs
311 33%, respectively, $P=0.6$). As shown in Fig. 5, neither the antiviral effectivenesses of PIs nor
312 that of Peg-IFN was significantly associated with the long term virological response. However
313 the loss rate of infected cells, δ , was significantly higher in patients that subsequently
314 achieved SVR (median $\delta^{SVR} = 0.27 \text{ day}^{-1}$ vs median $\delta^{non-SVR} = 0.14 \text{ day}^{-1}$, $P=0.03$).

315 Lastly we verified that incorporating the effect of RBV exposure in the PK-VK model, either
316 on the block of viral production or in the decrease of viral infectivity (data not shown) did not
317 improve the fit of the data. Furthermore there was no significant association between the
318 predicted C_{ss}^{RBV} and long term virological response ($P=0.5$).

319

320 **Discussion**

321 Here we used a PK-VK model to provide the first detailed picture of the relationship between
322 the exposure to all drugs involved in triple therapy (Peg-IFN, RBV and telaprevir or
323 boceprevir) and the early virological response. This novel model provides important insights
324 into the understanding of the response to triple therapy in hard-to treat patients.

325 We predicted that both PIs achieved a high level of antiviral effectiveness in blocking viral
326 production that was higher than 97.9% in all patients. However telaprevir had a higher
327 intrinsic potency than boceprevir, as measured by EC_{50} ($P=0.008$ after correcting for small
328 sample size), leading to a significantly higher level of antiviral effectiveness than boceprevir
329 ($\epsilon_{ss}^{telaprevir} = 99.8\%$ vs $\epsilon_{ss}^{boceprevir} = 99.0\%$, $P=0.002$) *i.e.* a 5-fold difference in the viral
330 production under treatment. Importantly the difference in EC_{50} was obtained despite the fact
331 that the study was not randomized and that patients who received telaprevir had less favorable
332 baseline characteristics than those who received boceprevir with higher baseline VL (6.43
333 \log_{10} IU/ml vs 5.52 \log_{10} IU/ml, respectively, $P<10^{-4}$) and a higher proportion of null
334 responder to previous bitherapy (4/9 vs 1/6).

335 The comparison of drug's antiviral effectiveness should be taken with caution because of
336 small sample size, the absence of randomization, and the fact that only trough concentrations
337 were used to estimate the EC_{50} of PI which may lead to underestimation. Yet these results
338 demonstrate for the first time a significant association between serum exposure to PI agents
339 and the antiviral effectiveness achieved. To confirm the significance of this association we
340 fitted HCV RNA data to a simplified model where drug exposure was not taken into account
341 (37). As compared to this model, we found that the PK-VK model both improved the fitting
342 criterion (BIC decreases from 181.3 to 176.3, *i.e.* an improvement of 5 points which is
343 regarded as positive evidence) and reduced the between-patient parameter variability by 26%

344 ($\omega_{EC_{50}PI}$ from 0.85 to 0.61), thus demonstrating that serum PK is an important predictor of the
345 antiviral effectiveness of triple therapy.

346 Our estimate that telaprevir achieves an antiviral effectiveness of 99.8% is largely similar to
347 the one found in naïve patients (15), suggesting that compensated cirrhosis does not affect the
348 maximal antiviral effectiveness of telaprevir. Whether this is also true for boceprevir is not
349 known as to our knowledge there is no published viral kinetic modeling study evaluating the
350 *in vivo* antiviral effectiveness of boceprevir.

351 In contrast to the high effectiveness achieved by both PIs, Peg-IFN was found to have a
352 modest contribution in blocking viral production, with a mean value of 43.4%. Of note
353 including the patient who did not receive Peg-IFN in our analysis allow us to add information
354 on telaprevir antiviral effectiveness. Further RBV exposure had no significant contribution on
355 the early viral kinetics. Together these results indicate that Peg-IFN and RBV have a minimal
356 contribution on the early virologic response, at least on this population of previous non-
357 responders to a Peg-IFN/RBV therapy.

358 In order to achieve a rapid viral decline, it is important to achieve not only a high level of
359 effectiveness but also a rapid second phase of viral decline. Here the latter was rather slow in
360 both treatment groups compared to what had been than found in telaprevir treated patients,
361 and this was attributed in our model to a low loss rate of infected cells, δ , about three times
362 smaller than in non-cirrhotic naive-patients (δ of 0.18 day⁻¹ vs 0.60 day⁻¹) (15, 16). Those
363 lower values may encompass several factors, such a lower penetration of PIs into infected
364 cells in a highly scarced liver. Because the loss rate of infected cells is strongly related to the
365 treatment duration needed to achieve SVR (15), our results suggest that the time to achieve
366 SVR in this population could be longer than what had been predicted from clinical trials (15).
367 Consistent with this prediction, the relapse rate in the CUPIC trial was equal to 41% in both

368 treatment groups (13), *i.e.*, much higher than what reported in treatment experienced patients
369 phase 3 clinical trials (12% to 27%) (9, 11, 22).

370 Regarding the use of early viral kinetic parameters for treatment prediction, we found that δ
371 was higher in patients that subsequently achieved SVR (median $\delta^{SVR} = 0.27 \text{ day}^{-1}$ vs median
372 $\delta^{non-SVR} = 0.14 \text{ day}^{-1}$, $P=0.03$) suggesting that δ could be a relevant predictor of the outcome of
373 triple therapy, as it was the case for Peg-IFN/RBV bitherapy (38). In contrast there was no
374 significant relationship between antiviral effectiveness of PIs on SVR (Fig. 6A). This absence
375 of relationship is consistent with the hypothesis that in order to achieve SVR, it is necessary
376 not only to have a high antiviral effectiveness at treatment initiation, when the viral
377 population is predominantly wild-type and drug-sensitive, but also at later times, when the
378 viral population is predominantly resistant to PI agents (39, 40). The fact that neither Peg-IFN
379 effectiveness nor RBV were associated with SVR is more surprising, as one would expect
380 these agents to be equally active against wild-type and resistant virus. However our patient
381 population was both treatment experienced and cirrhotic, two major causes of insensitivity to
382 Peg-IFN/RBV.

383 Clearly the main limitation of this study was its small size. In a previous study we evaluated
384 by simulation the power to detect a difference of antiviral effectiveness between two
385 treatment groups for a variety of designs (34). With a design comparable to the present study,
386 *i.e.*, 10 patients per group, 7 VL per patient and an antiviral effectiveness of 99% vs 99.9%,
387 the power to detect this difference was 100% with the same statistical method that we used in
388 this analysis. Yet, further studies on larger populations will still be needed to estimate more
389 precisely the exposure-effect relationship (Fig. 4) and other kinetic parameters involved on
390 the long-term virologic response. A second limitation is that only trough pre-dose drug
391 concentrations were collected and modeled. Thus C_{ss} is the steady-state C_{trough} . Moreover no
392 information was collected on treatment adherence. The data analysis did not show any signal

393 of lack of adherence such as viral oscillations, which indicates that missed doses, if they
394 occurred, did not have a major effect on the observed kinetic of decline. Here we considered
395 that concentrations of PIs were constant over time. Detailed pharmacokinetic analysis showed
396 that steady state of residual concentrations is attained after two days of treatment (41). As
397 explained in details in Guedj *et al.* (42), the fact that we neglected this initial build up may
398 explain why our estimate of the viral clearance rate, c , was lower than previously found in
399 treatment naïve patients (15). Further the lack of information on the time of Peg-IFN injection
400 also precluded a precise characterization between Peg-IFN exposure and the virological
401 response. The fact that we used rather empirical models is less problematic for RBV, whose
402 long elimination half-life resulting in a slow increase over time could be well characterized
403 here (27). Moreover, as mentioned previously, in order to achieve SVR, it is important for
404 drugs to achieve a higher effectiveness against PI-resistant virus. Because no sequencing was
405 done here, we focused only the early virological response where presumably the virus is
406 predominantly drug-sensitive. In order to estimate PI effectiveness against resistant virus it
407 would be needed to quantify and follow the proportion of resistant virus over time, as early as
408 possible, for instance using pyrosequencing (43).

409 A greater proportion of patients that received boceprevir were genotype 1a relative to those
410 that received telaprevir ($P=0.04$). It has been well established that subgenotype is an important
411 predictor of the response to treatment and for instance the fact that telaprevir has a higher
412 genetic barrier to resistance with genotype 1b than 1a (36) may explain why genotype non-1a
413 patients were preferentially treated with telaprevir than boceprevir. However the effect of
414 subgenotype on the early viral kinetics, where most of the virus is drug-sensitive is unknown,
415 and has never been investigated as far as we know. In our study no significant effect of
416 subgenotype on any of the parameters (c , δ , EC_{50}^{PI}) was found.

417 The effect of RBV was analyzed using serum drug concentrations. Some authors preferably
418 used erythrocyte RBV concentration (44), which was not measured in the present study.
419 However a significant relationship was shown between erythrocyte RBV concentrations and
420 serum concentrations (45), suggesting that serum RBV can be used for the assessment of early
421 and sustained virological responses (46, 47).

422 To summarize this study provides the first characterization of the relationship between drug
423 concentrations involved in triple therapy and early HCV viral kinetics treated with telaprevir
424 or boceprevir. We found that median values of antiviral effectiveness for telaprevir was
425 similar to what had been found in treatment naïve patients and significantly larger than in
426 boceprevir treated patients. In all patients the second phase of viral decline was slow and may
427 explain the high relapse rate observed in the ANRS-CO20-CUPIC cohort. This suggests that,
428 notwithstanding safety issues, longer treatment duration could improve the treatment efficacy
429 and lead to a higher SVR rate. Lastly the antiviral effectiveness of Peg-IFN was modest (less
430 than 50%) suggesting that cirrhotic treatment experienced-patients may particularly benefit
431 from upcoming IFN-free treatment. Our approach, which shows the importance of PK data to
432 disentangle the effects of drug combination and to understand the variability in the virological
433 response, is not specific to triple therapy and could also be used to optimize future IFN-free
434 regimen, in particular in hard-to-treat patients.

435

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446

447 **Author Contributions:** CL, FM, and JG made the analysis and drafted the manuscript; all
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449

450 **Disclosure statement**

451 JG: has consulted with Gilead SC.

452 FZ: received speakers/consulting fees from Gilead SC, MSD, BMS, Janssen cilag, Abbvie,
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461

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- 627

628 **Figure legends**

629

630 **Fig. 1: Observed concentrations over time.**

631 (a) telaprevir in 9 patients (black, $\mu\text{mol/ml}$) and boceprevir in 6 six patients (grey, $\mu\text{mol/ml}$);
632 (b) Peg-IFN in telaprevir group (black, ng/ml) and in boceprevir group (grey, ng/ml); (c) RBV
633 in telaprevir group (black, ng/ml) and in boceprevir group (grey, ng/ml). Patients who
634 received a boceprevir-based therapy had only two blood samples during the lead-in phase at
635 baseline and week 2.

636

637 **Fig. 2: Individual fits of the viral decline (\log_{10} IU/ml).**

638 Nine patients in telaprevir group (black curve) and 6 patients in boceprevir group (grey
639 curve). Black crosses represent the observed viral load and grey stars represent the viral load
640 under the limit of detection.

641

642 **Fig. 3. Goodness-of-fit of the viral kinetic-pharmacokinetic model**

643 Residuals (weighted residuals calculated using individual predictions: IWRES and normalized
644 prediction distribution errors: NPDE) versus time and versus predictions plots. Residuals
645 seem to distribute homogenously around 0.

646 Observed viral load are plotted as black crosses and viral load under the limit of detection as
647 grey stars.

648

649 **Fig. 4. Relationship between predicted trough concentration at steady state (C_{ss}) and**
650 **predicted antiviral effectivenesses (ϵ_{ss}).**

651 (a) for the protease inhibitor (telaprevir in black and boceprevir in grey, $\mu\text{mol/l}$); (b) for Peg-
652 IFN (Peg-IFN- α 2a in black and Peg-IFN- α 2b in grey, ng/ml). The lines denote the predictions

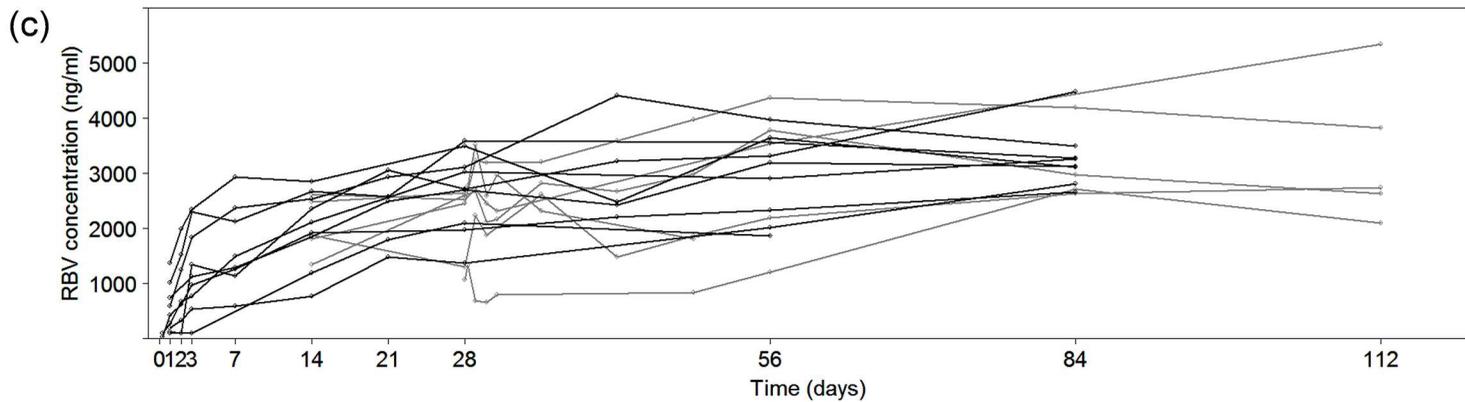
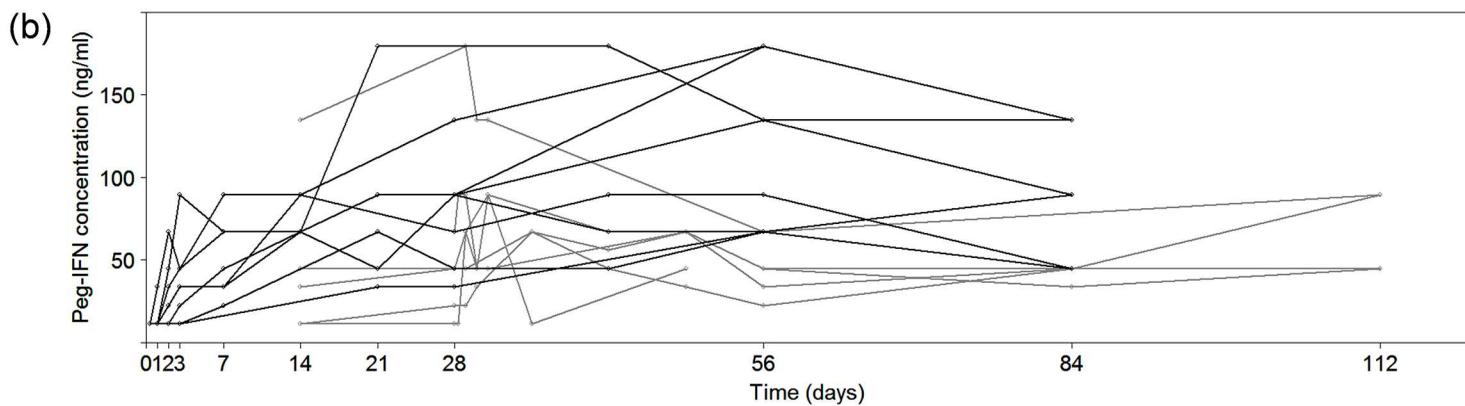
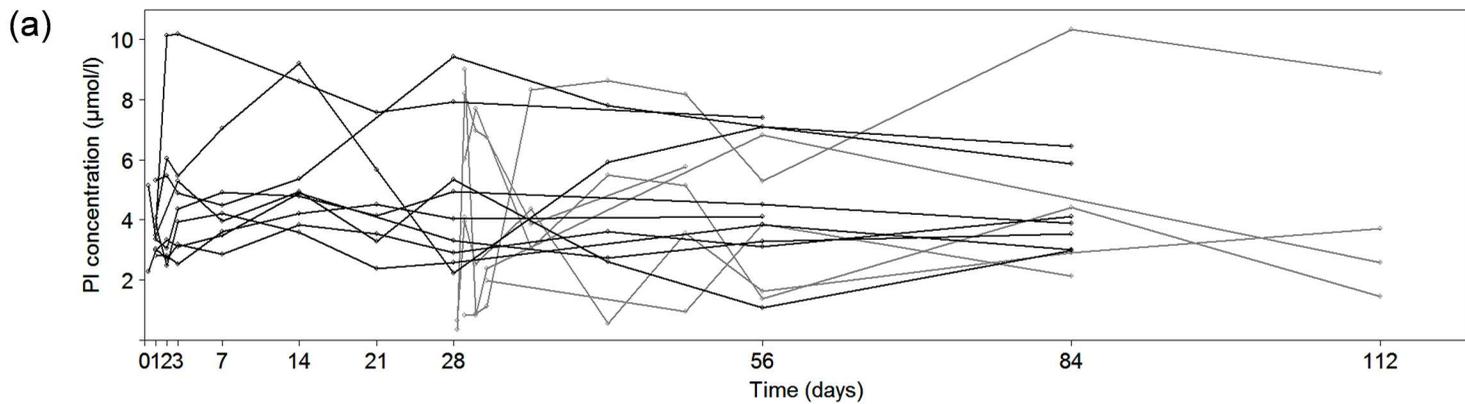
653 with the mean antiviral effectiveness and the dotted lines denote 95% confidence interval
654 computed with the standard errors predicted by the Fisher Information Matrix.

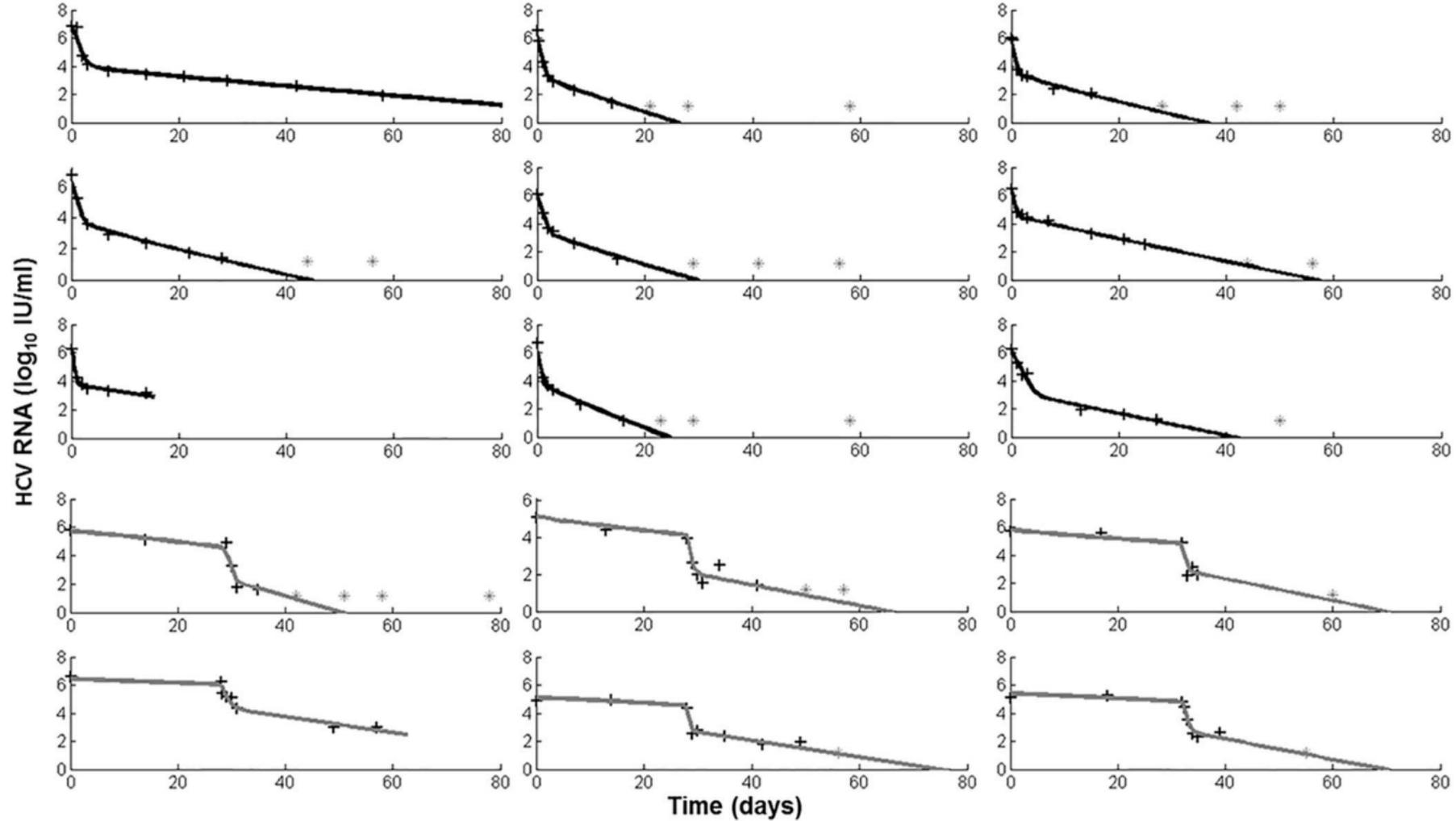
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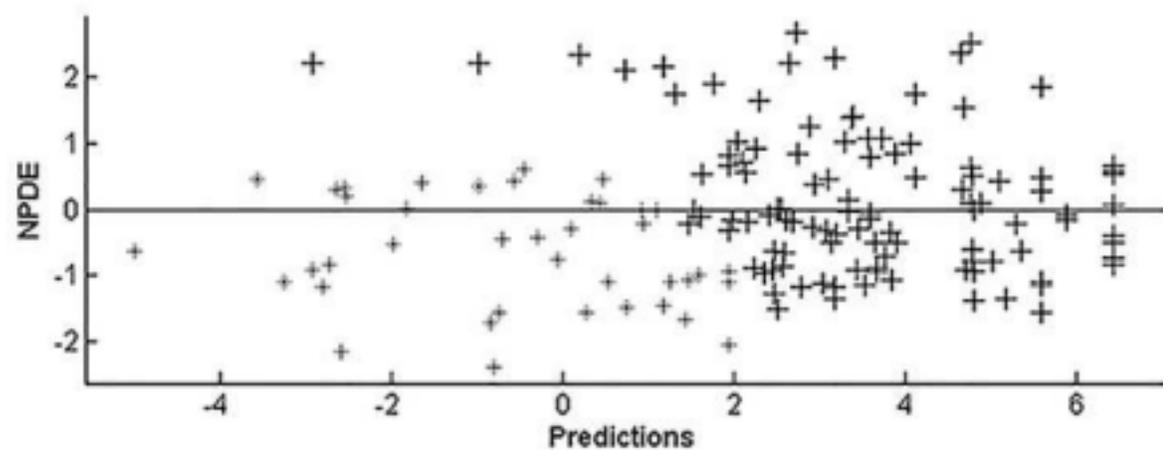
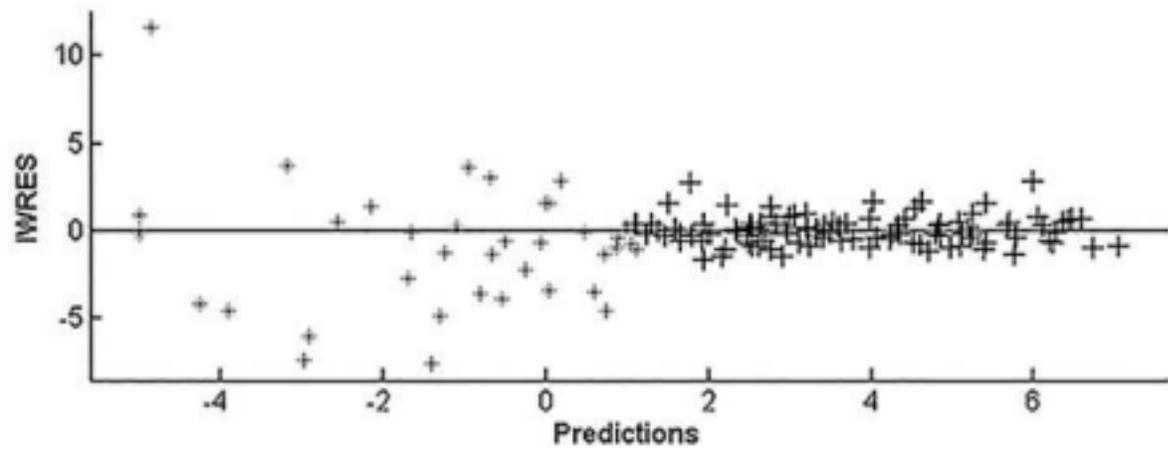
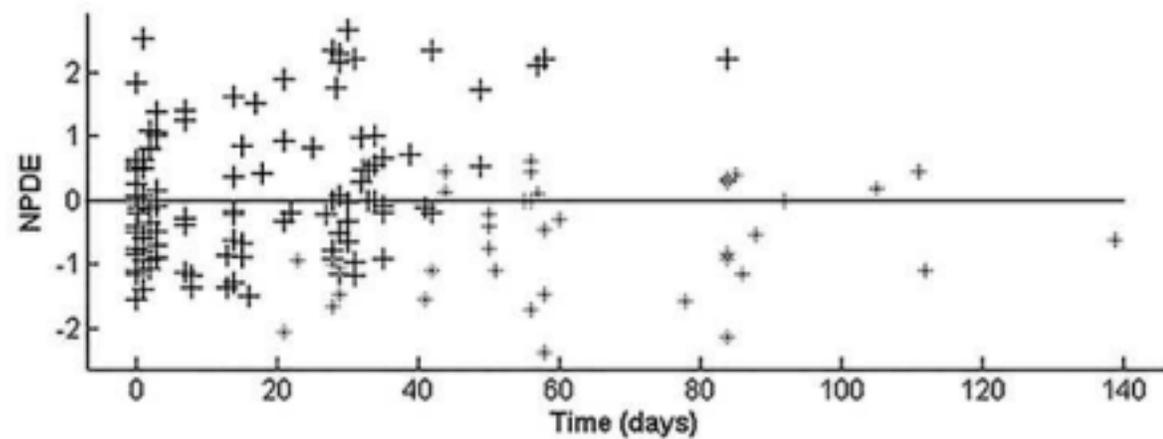
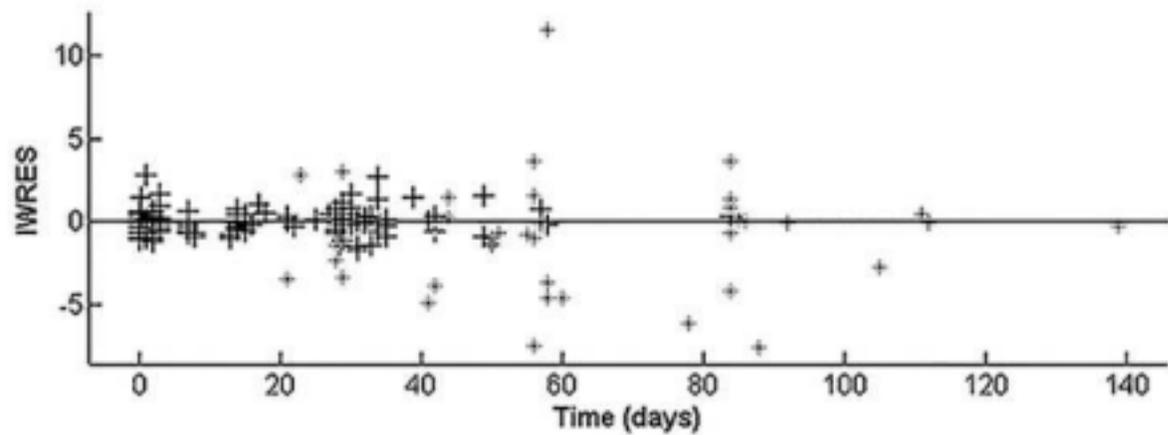
656 **Fig. 5: Relationship between long term virological response (SVR) and parameters**
657 **estimated by the viral kinetic-pharmacokinetic model.**

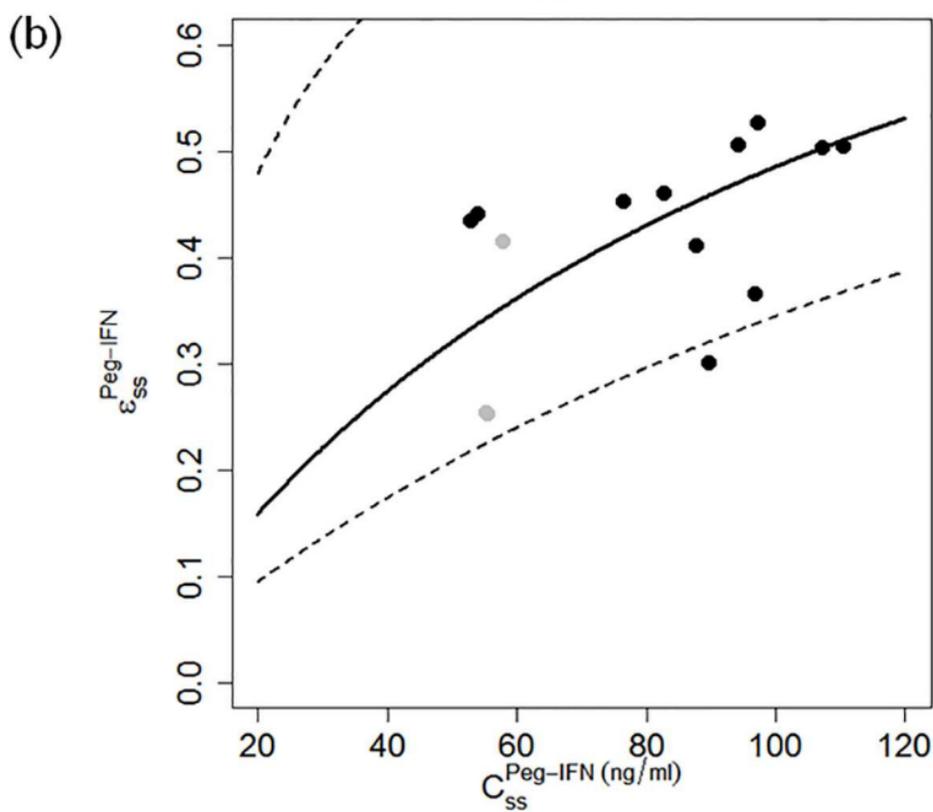
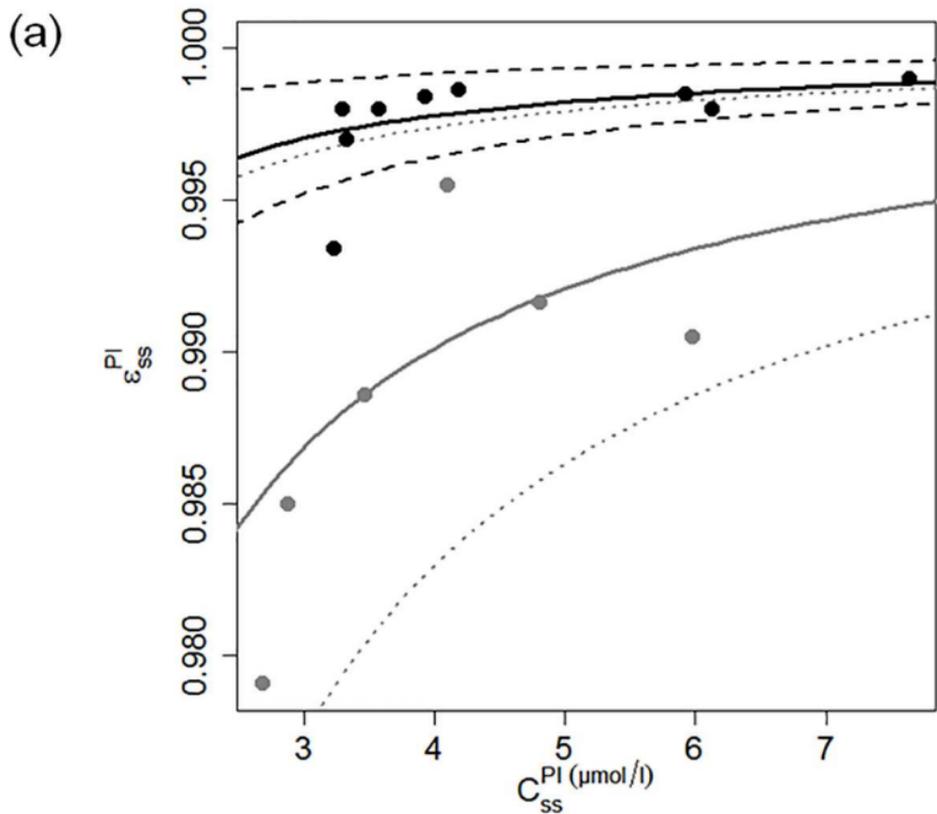
658 (a) predicted antiviral effectivenesses (ϵ_{ss}) of PIs; (b) predicted antiviral effectivenesses (ϵ_{ss})

659 of Peg-IFN; (c) δ delta parameter (loss rate of infected cells). P-value from Wilcoxon tests.









1 **Table 1. Main patient characteristics**

| | Peg-IFN/RBV + telaprevir | Peg-IFN/RBV + boceprevir | Total |
|---|-------------------------------------|-------------------------------------|---------------|
| | n=9 | n=6 | n=15 |
| Age (years), median [min-max] | 55 [49-59] | 53 [44-64] | 55 [44-64] |
| Males, n (%) | 8 (89) | 4 (67) | 12 (80) |
| HCV RNA (log ₁₀ IU/ml), median [min-max] | 6.5 [6.0-6.8] | 5.4 [4.9-6.6] | 6.2 [4.9-6.8] |
| HCV genotype, n (%): | | | |
| 1a | 2 (22) | 5 (83) | 7 (47) |
| Non 1a | 7 (78) | 1 (17) | 8 (53) |
| IL28B genotype (rs12979860), n (%): | | | |
| C/C | 2 (22) | - | 2 (13) |
| C/T | 6 (67) | 6 (100) | 12 (80) |
| T/T | 1 (11) | - | 1 (7) |
| Response to previous bitherapy, n (%): | | | |
| Partial responder | - | 2 (33) | 2 (13) |
| Null responder | 4 (44) | 1 (17) | 5 (33) |
| Relapser | 3 (33) | 3 (50) | 6 (40) |
| Early discontinuation for adverse event | 2 (22) | - | 2 (13) |

2

3

4 **Table 2. Individual predicted trough concentrations at steady state (C_{ss})**

| | n | median [min; max] |
|--|----|----------------------|
| $C_{ss}^{\text{telaprevir}}$ ($\mu\text{mol/l}$) | 9 | 3.77 [2.68; 5.98] |
| $C_{ss}^{\text{boceprevir}}$ ($\mu\text{mol/l}$) | 6 | 3.92 [3.22; 7.64] |
| $C_{ss}^{\text{Peg-IFN-}\alpha 2a}$ (ng/ml) | 11 | 89.6 [52.8; 110.4] |
| $C_{ss}^{\text{Peg-IFN-}\alpha 2b}$ (ng/ml) | 3 | 55.4 [55.3; 57.9] |
| C_{ss}^{RBV} (ng/ml) | 15 | 2,860 [2,428; 3,874] |

5

6 **Table 3. Parameter estimates and relative standard errors (RSE)**

| | Estimate | RSE (%) |
|---|-----------------|----------------|
| $V_0^{\text{telaprevir}}$ (\log_{10} IU/ml) | 6.43 | 2 |
| $V_0^{\text{boceprevir}}$ (\log_{10} IU/ml) | 5.52 | 3 |
| c (day^{-1}) | 3.98 | 12 |
| δ (day^{-1}) | 0.18 | 11 |
| $EC_{50}^{\text{Peg-IFN}}$ (ng/ml) | 106 | 40 |
| $EC_{50}^{\text{telaprevir}}$ ($\mu\text{mol/l}$) | 0.009 | 30 |
| $EC_{50}^{\text{boceprevir}}$ ($\mu\text{mol/l}$) | 0.04 | 43 |
| ω_{V_0} | 0.07 | 20 |
| ω_c | 0.47 | 19 |
| ω_δ | 0.42 | 16 |
| $\omega_{EC_{50}^{\text{Peg-IFN}}}$ | 0.67 | 30 |
| $\omega_{EC_{50}^{\text{PI}}}$ | 0.61 | 32 |
| σ | 0.27 | 7 |

7 V_0 : baseline viral load; c : clearance rate of virus from serum; δ : loss rate of
8 infected cells; EC_{50} : half maximal effective concentration; ω : inter-
9 individual variability; σ : standard deviation of residual error; RSE: relative
10 standard errors of parameter estimates, PI: protease inhibitor.