



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.

Cédric Laouénan, Patrick Marcellin, Martine Lapalus, Feryel Khelifa-Mouri, Nathalie Boyer, Fabien Zoulim, Lawrence Serfaty, Jean-Pierre Bronowicki, Michelle Martinot-Peignoux, Olivier Lada, et al.

► To cite this version:

Cédric Laouénan, Patrick Marcellin, Martine Lapalus, Feryel Khelifa-Mouri, Nathalie Boyer, et al.. 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.: Effectiveness of triple therapy in cirrhotic patients. *Antimicrobial Agents and Chemotherapy*, 2014, 58 (9), pp.5332-41. 10.1128/AAC.02611-14 . inserm-01059165

HAL Id: inserm-01059165

<https://inserm.hal.science/inserm-01059165>

Submitted on 29 Aug 2014

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Cédric Laouénan,^{a,b,#} Patrick Marcellin,^{c,d} Martine Lapalus,^c Feryel Khelifa-Mouri,^d Nathalie Boyer,^d Fabien Zoulim,^{e,f} Lawrence Serfaty,^g Jean-Pierre Bronowicki,^{h,i} Michelle Martinot-Peignoux,^c Olivier Lada,^c Tarik Asselah,^{c,d} Céline Dorival,^j Christophe Hézode,^{k,l} Fabrice Carrat,^{j,m} Florence Nicot,ⁿ Gilles Peytavin,^{a,o} France Mentré,^{a,b} Jeremie Guedj^a.

INSERM, IAME, UMR 1137, Univ Paris Diderot, Sorbonne Paris Cité, Paris, France^a; AP-HP, Hôpital Bichat, Département of Biostatistic, Paris, France^b; INSERM, CRI Paris Montmartre, UMR 1149, Univ Paris Diderot, Clichy, France^c; AP-HP, Hôpital Beaujon, Hepatology, Physiopathology and Treatment of Viral Hepatitis, Clichy, France^d; INSERM, UMR 1052, Univ Lyon, Lyon, France^e; Hospices Civils de Lyon, Department of Hepatology, Lyon, France^f; AP-HP, Hôpital Saint-Antoine, Department of Hepatology, Paris, France^g; INSERM, UMR 954, Univ Lorraine, Vandoeuvre-les-Nancy, France^h; Centre Hospitalier Universitaire de Nancy, Department of Hepatology, Vandoeuvre-les-Nancy, Franceⁱ; INSERM, UMR 707, Univ Pierre et Marie Curie, Paris, France^j; INSERM, UMR 955, Univ Paris-Est, Créteil, France^k; AP-HP, Hôpital Henri Mondor, Department of Hepatology, Créteil, France^l; AP-HP, Hôpital Saint-Antoine, Department of public health, Paris, France^m; CHU Toulouse, IFB Purpan, Virology, laboratory, Toulouse, Franceⁿ; AP-HP, Hôpital Bichat, Department of Clinical Pharmacokinetics, Paris, France^o.

Running Head: Effectiveness of triple therapy in cirrhotic patients

26 # Address correspondence to Cédric Laouénan, cedric.laouenan@inserm.fr

27

28 **Word count:**

29 - Abstract: 243

30 - Article: 4685

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

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

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

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

Drug pharmacokinetic modeling

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

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

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

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

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

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

Viral kinetic modeling

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

$$\begin{aligned} \frac{dI}{dt} &= bVT - \delta I \\ \frac{dV}{dt} &= p(1 - \varepsilon(t))I - cV \end{aligned} \quad \text{Eq. (4)}$$

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

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

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

$$\varepsilon^{PI}(t) = \frac{C^{PI}(t)}{C^{PI}(t) + EC_{50}^{PI}}$$

$$\varepsilon^{Peg-IFN}(t) = \frac{C^{Peg-IFN}(t)}{C^{Peg-IFN}(t) + EC_{50}^{Peg-IFN}} \quad \text{Eq. (5)}$$

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

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

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

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

Data analysis and parameter estimation

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

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

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

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

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

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

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

Prediction and comparison of individual parameters

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

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

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

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

Results

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

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

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

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

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

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

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

After PI initiation, VL declines in a biphasic manner in all patients, where a rapid first phase was followed by a second slower phase. The rapid first phase was attributed to a clearance rate of virus, c , equal to 3.98 day^{-1} and to a high level of antiviral effectivenesses for both PIs.

The intrinsic potency of the two molecules, as measured by the EC_{50}^{PI} , was significantly higher for telaprevir than boceprevir ($EC_{50}^{telaprevir} = 0.009 \text{ } \mu\text{mol/l}$ vs $EC_{50}^{boceprevir} = 0.04 \text{ } \mu\text{mol/l}$, $P=0.008$). Importantly the statistical significance of this difference was obtained after taking into account the small sample size (see methods) and adjusted on baseline VL. Since telaprevir had a lower EC_{50} than boceprevir and that both drugs achieved similar levels of molar concentrations the model predicted that the median individual antiviral effectiveness of

PI agent in blocking viral production was significantly higher in patients that received
 telaprevir than in those who received boceprevir ($\varepsilon_{ss}^{telaprevir} = 99.8\% [99.3; 99.9]$ and $\varepsilon_{ss}^{boceprevir}$
 $= 99.0\% [98.0; 99.6]$, $P=0.002$). Interestingly this model could well capture the relationship
 between the serum exposure and its antiviral effectiveness, demonstrating that the variability
 in drug exposure needs to be taken into account to understand the between-subject variability
 in PIs antiviral effectiveness (Fig. 4A). Lastly because the effectiveness of both PIs were
 much larger than that of Peg-IFN (Fig. 4B), the total antiviral effectiveness obtained by the
 combination of PI and Peg-IFN was largely similar to the one obtained with the PIs only.
 After the VL was rapidly reduced as a result of the strong antiviral effectiveness of both PIs,
 the model predicted that a second slower phase of viral decline ensued, driven by the loss rate
 of infected cells, δ . We estimated δ to be equal to 0.18 day^{-1} , corresponding to a half-life of
 infected cells of 3.9 days, with no significant differences between patients receiving telaprevir
 and boceprevir ($P=0.5$).
 Next we investigated the relationship between the PK-VK parameters and SVR. Among the 7
 patients (47%) who achieved SVR, 5 received telaprevir and 2 received boceprevir (56% vs
 33%, respectively, $P=0.6$). As shown in Fig. 5, neither the antiviral effectivenesses of PIs nor
 that of Peg-IFN was significantly associated with the long term virological response. However
 the loss rate of infected cells, δ , was significantly higher in patients that subsequently
 achieved SVR (median $\delta^{SVR} = 0.27 \text{ day}^{-1}$ vs median $\delta^{non-SVR} = 0.14 \text{ day}^{-1}$, $P=0.03$).
 Lastly we verified that incorporating the effect of RBV exposure in the PK-VK model, either
 on the block of viral production or in the decrease of viral infectivity (data not shown) did not
 improve the fit of the data. Furthermore there was no significant association between the
 predicted C_{ss}^{RBV} and long term virological response ($P=0.5$).

Discussion

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

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

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

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

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

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

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

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

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

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

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

A greater proportion of patients that received boceprevir were genotype 1a relative to those that received telaprevir ($P=0.04$). It has been well established that subgenotype is an important predictor of the response to treatment and for instance the fact that telaprevir has a higher genetic barrier to resistance with genotype 1b than 1a (36) may explain why genotype non-1a patients were preferentially treated with telaprevir than boceprevir. However the effect of subgenotype on the early viral kinetics, where most of the virus is drug-sensitive is unknown, and has never been investigated as far as we know. In our study no significant effect of 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

Acknowledgements

The study was sponsored and funded by The National Agency for research on Aids and viral Hepatitis (ANRS) and in part by the Association Française pour l'Etude du Foie (AFEF). Sponsors had no role in interpretation of data, in the writing of the report, or in the decision to submit the paper for publication.

The authors thank Ventzislava Petrov Sanchez and Setty Allam (from unit Basic and Clinical research on viral hepatitis, French National Agency for research on Aids and viral Hepatitis, Paris, France) and Cécilie Dufour (from Inserm UMR 707, University Pierre et Marie Curie, Paris, France). The authors thank Dr Marie Anne Loriot (from Inserm UMR 1147, University Paris Descartes, Paris, France) for genotyping the IL28B rs12979860 polymorphism.

Author Contributions: CL, FM, and JG made the analysis and drafted the manuscript; all authors provided the data; all authors read and approved the final manuscript.

Disclosure statement

JG: has consulted with Gilead SC.

FZ: received speakers/consulting fees from Gilead SC, MSD, BMS, Janssen cilag, Abbvie, Boehringer Ingelheim.

CH: has been a clinical investigator, speaker and/or consultant for Abbvie, Boehringer Ingelheim, BMS, Gilead Sciences, Janssen, Merck Sharp & Dohme, and Roche.

PM: has been a clinical investigator, speaker and/or consultant for Roche, Gilead, Vertex, Novartis, Janssen - Tibotec, MSD, Boehringer, Abbott, Pfizer, Alios BioPharma.

GP: has received travel grants, consultancy fees, honoraria or study grants from various pharmaceutical companies, including Bristol-Myers-Squibb, Gilead SC, Janssen, Merck, ViiV Healthcare and Splicos.

References

1. World Health Organization. WHO Fact Sheet 164-Hepatitis C. Available at: <http://www.who.int/gate2.inist.fr/mediacentre/factsheets/fs164/en/>. Accessed Januray 31, 2014.
2. **Shepard CW, Finelli L, Alter, MJ.** 2005. Global epidemiology of hepatitis C virus infection. *Lancet Infect. Dis.* **5**:558–567.
3. **Pearlman BL.** 2012. Protease inhibitors for the treatment of chronic hepatitis C genotype-1 infection: the new standard of care. *Lancet Infect. Dis.* **12**:717–728.
4. **Poordad F, McCone J Jr, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki J-P.** 2011. Boceprevir for untreated chronic HCV genotype 1 infection. *N. Engl. J. Med.* **364**:1195–1206.
5. **McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, Alam J, Muir AJ.** 2009. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N. Engl. J. Med.* **360**:1827–38.
6. **Asselah T, Marcellin P.** 2014. Second-wave IFN-based triple therapy for HCV genotype 1 infection: simeprevir, faldaprevir and sofosbuvir. *Liver Int.* **34**:60–68.
7. **Deuffic-Burban S, Schwarzhinger M, Obach D, Mallet V, Pol S, Pageaux GP, Canva V, Deltenre P, Roudot-Thoraval F, Larrey D, Dhumeaux D, Mathurin P, Yazdanpanah Y.** 2014. Should we await IFN-free regimens to treat HCV genotype 1 treatment-naïve patients? A cost-effectiveness analysis (ANRS 12188). *J. Hepatol.*, in press.
8. **Flamm SL, Lawitz E, Jacobson I, Bourlière M, Hezode C, Vierling JM, Bacon BR, Niederau C, Sherman M, Goteti V, Sings HL, Barnard RO, Howe JA, Pedicone LD, Burroughs MH, Brass CA, Albrecht JK, Poordad F.** 2013. Boceprevir with peginterferon alfa-2a-ribavirin is effective for previously treated chronic hepatitis C genotype 1 infection. *Clin. Gastroenterol. Hepatol.* **11**:81–87.
9. **Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A, Ferenci P, Nevens F, Müllhaupt B, Pockros P, Terg R, Shouval D, van Hoek B, Weiland O, Van Heeswijk R, De Meyer S, Luo D, Boogaerts G, Polo R, Picchio G, Beumont M, REALIZE Study Team.** 2011. Telaprevir for retreatment of HCV infection. *N. Engl. J. Med.* **364**:2417–2428.

10. **McHutchison JG, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, Heathcote EJ, Zeuzem S, Reesink HW, Garg J, Bsharat M, George S, Kauffman RS, Adda N, Di Bisceglie AM.** 2010. Telaprevir for previously treated chronic HCV infection. *N. Engl. J. Med.* **362**:1292–303.
11. **Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R.** 2011. Boceprevir for previously treated chronic HCV genotype 1 infection. *N. Engl. J. Med.* **364**:1207–1217.
12. **Hézode C, Fontaine H, Dorival C, Larrey D, Zoulim F, Canva V, de Ledinghen V, Poynard T, Samuel D, Bourlière M, Zarski JP, Raabe JJ, Alric L, Marcellin P, Riachi G, Bernard PH, Loustaud-Ratti V, Métivier S, Tran A, Serfaty L, Abergel A, Causse X, Di Martino V, Guyader D, Lucidarme D, Grando-Lemaire V, Hillon P, Feray C, Dao T, Cacoub P, Rosa I, Attali P, Petrov-Sanchez V, Barthe Y, Pawlotsky JM, Pol S, Carrat F, Bronowicki JP; CUPIC Study Group.** 2013. Triple therapy in treatment-experienced patients with HCV-cirrhosis in a multicentre cohort of the French Early Access Programme (ANRS CO20-CUPIC). *J. Hepatol.* **59**:434-441.
13. **Hézode C, Fontaine H, Dorival C, Zoulim F, Larrey D, Canva V, de Ledinghen V, Poynard T, Samuel D, Bourlière M, Alric L, Raabe J-J, Zarski J-P, Marcellin P, Riachi G, Bernard P-H, Loustaud-Ratti V, Chazouilleres O, Abergel A, Guyader D, Metivier S, Tran A, di Martino V, Causse X, Dao T, Lucidarme D, Portal I, Cacoub P, Gournay J, Grando-Lemaire V, Hillon P, Attali P, Fontanges T, Rosa I, Petrov-Sanchez V, Barthe Y, Pawlotsky J-M, Pol S, Carrat F, Bronowicki J-P, the cupic study group.** 2014. Effectiveness of Telaprevir or Boceprevir in Treatment-experienced Patients with HCV Genotype 1 Infection and Cirrhosis. *Gastroenterology*, in press.
14. **Chatterjee A, Guedj J, Perelson AS.** 2012. Mathematical modelling of HCV infection: what can it teach us in the era of direct-acting antiviral agents? *Antivir. Ther.* **17**:1171–1182.
15. **Guedj J, Perelson AS.** 2011. Second-phase hepatitis C virus RNA decline during telaprevir-based therapy increases with drug effectiveness: implications for treatment duration. *Hepatology* **53**:1801-1808.
16. **Adiwijaya BS, Kieffer TL, Henshaw J, Eisenhauer K, Kimko H, Alam JJ, Kauffman RS, Garg V.** 2012. A viral dynamic model for treatment regimens with direct-acting antivirals for chronic hepatitis C infection. *PLoS Comput. Biol.* **8**(1):e1002339.

17. **Adiwijaya BS, Herrmann E, Hare B, Kieffer T, Lin C, Kwong AD, Garg V, Randle JC, Sarrazin C, Zeuzem S, Caron PR.** 2010. A multi-variant, viral dynamic model of genotype 1 HCV to assess the in vivo evolution of protease-inhibitor resistant variants. *PLoS Comput. Biol.* **6**(4):e1000745.
18. **Fontaine H, Petitprez K, Roudot-Thoraval F, Trinchet J-C.** 2007. Guidelines for the diagnosis of uncomplicated cirrhosis. *Gastroenterol. Clin. Biol.* **31**:504–509.
19. **Farnik H, El-Duweik J, Welsch C, Sarrazin C, Lötsch J, Zeuzem S, Geisslinger G, Schmidt H.** 2009. Highly sensitive determination of HCV protease inhibitors boceprevir (SCH 503034) and telaprevir (VX 950) in human plasma by LC-MS/MS. *J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci.* **877**:4001–4006.
20. **Homma M, Jayewardene AL, Gambertoglio J, Aweeka F.** 1999. High-performance liquid chromatographic determination of ribavirin in whole blood to assess disposition in erythrocytes. *Antimicrob. Agents Chemother.* **43**:2716–2719.
21. **Boulestin A, Kamar N, Legrand-Abravanel F, Sandres-Saune K, Alric L, Vinel J-P, Rostaing L, Izopet J.** 2004. Convenient biological assay for polyethylene glycol-interferons in patients with hepatitis C. *Antimicrob. Agents Chemother.* **48**:3610–3612.
22. **Kieran J, Schmitz S, O’Leary A, Walsh C, Bergin C, Norris S, Barry M.** 2013. The relative efficacy of boceprevir and telaprevir in the treatment of hepatitis C virus genotype 1. *Clin. Infect. Dis.* **56**:228–235.
23. **Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, Perelson AS.** 1998. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science* **282**:103–7.
24. **Guedj J, Rong L, Dahari H, Perelson AS.** 2010. A perspective on modelling hepatitis C virus infection. *J. Viral Hepat.* **17**:825–833.
25. **Shudo E, Ribeiro RM, Talal AH, Perelson AS.** 2008. A hepatitis C viral kinetic model that allows for time-varying drug effectiveness. *Antivir. Ther.* **13**:919–926.
26. **Rong L, Dahari H, Ribeiro RM, Perelson AS.** 2010. Rapid emergence of protease inhibitor resistance in hepatitis C virus. *Sci. Transl. Med.* **2**:30ra32.
27. **Pawlotsky J-M, Dahari H, Neumann AU, Hezode C, Germanidis G, Lonjon I, Castera L, Dhumeaux D.** 2004. Antiviral action of ribavirin in chronic hepatitis C. *Gastroenterology* **126**:703–714.
28. **Rotman Y, Nouredin M, Feld JJ, Guedj J, Witthaus M, Han H, Park YJ, Park S-H, Heller T, Ghany MG, Doo E, Koh C, Abdalla A, Gara N, Sarkar S, Thomas E, Ahlenstiel G, Edlich B, Titerence R, Hogdal L, Rehmann B, Dahari H, Perelson**

- AS, Hoofnagle JH, Liang TJ. 2014. Effect of ribavirin on viral kinetics and liver gene expression in chronic hepatitis C. *Gut* **63**:161–169.
29. Mihm U, Welker M-W, Teuber G, Wedemeyer H, Berg T, Sarrazin C, Böhm S, Alshuth U, Herrmann E, Zeuzem S. 2014. Impact of ribavirin priming on viral kinetics and treatment response in chronic hepatitis C genotype 1 infection. *J. Viral Hepat.* **21**:42–52.
30. Dixit NM, Layden-Almer JE, Layden TJ, Perelson AS. 2014. Modelling how ribavirin improves interferon response rates in hepatitis C virus infection. *Nature* **432**:922–924.
31. Samson A, Lavielle M, Mentre F. 2006. Extension of the SAEM algorithm to left censored data in nonlinear mixed-effects model: Application to HIV dynamics model. *Comput. Stat. Data Anal.* **51**:1562–1574.
32. Thiébaud R, Guedj J, Jacqmin-Gadda H, Chêne G, Trimoulet P, Neau D, Commenges D. 2006. Estimation of dynamical model parameters taking into account undetectable marker values. *BMC Med. Res. Methodol.* **6**:38.
33. Guedj J, Pang PS, Denning J, Rodriguez-Torres M, Lawitz E, Symonds W, Perelson AS. 2014. Analysis of the hepatitis C viral kinetics during administration of two nucleotide analogues: sofosbuvir (GS-7977) and GS-0938. *Antivir. Ther.* **19**:211–220.
34. Laouénan C, Guedj J, Mentré F. 2013. Clinical trial simulation to evaluate power to compare the antiviral effectiveness of two hepatitis C protease inhibitors using nonlinear mixed effect models: a viral kinetic approach. *BMC Med. Res. Methodol.* **13**:60.
35. Holmes JA, Desmond PV, Thompson AJ. 2012. Does IL28B genotyping still have a role in the era of direct-acting antiviral therapy for chronic hepatitis C infection? *J. Viral Hepat.* **19**:677–684.
36. Cunningham M, Foster GR. 2012. Efficacy and safety of telaprevir in patients with genotype 1 hepatitis C infection. *Ther. Adv. Gastroenterol.* **5**:139–151.
37. Laouénan C, Guedj J, Lapalus M, Khelifa-Mouri F, Martinot-Peignoux M, Boyer N, Serfaty L, Bronowicki JP, Zoulim F, Mentré F, Marcellin P. 2013. Characterization of the early viral kinetics in compensated cirrhotic treatment-experienced patients treated with boceprevir and telaprevir, abstr 08c. 48th annual meeting of the European Association for the Study of the Liver (EASL), Amsterdam, The Netherlands.

38. **Talal AH, Ribeiro RM, Powers KA, Grace M, Cullen C, Hussain M, Markatou M, Perelson AS.** 2006. Pharmacodynamics of PEG-IFN alpha differentiate HIV/HCV coinfectd sustained virological responders from nonresponders. *Hepatology* **43**:943–953.
39. **Halfon P, Locarnini S.** 2011. Hepatitis C virus resistance to protease inhibitors. *J. Hepatol.* **55**:192–206.
40. **Sarrazin C, Zeuzem S.** 2010. Resistance to direct antiviral agents in patients with hepatitis C virus infection. *Gastroenterology* **138**:447–462.
41. **Yamada I, Suzuki F, Kamiya N, Aoki K, Sakurai Y, Kano M, Matsui H, Kumada H.** 2012. Safety, pharmacokinetics and resistant variants of telaprevir alone for 12 weeks in hepatitis C virus genotype 1b infection. *J. Viral Hepat.* **19**:e112–119.
42. **Guedj J, Dahari H, Shudo E, Smith P, Perelson AS.** 2012. Hepatitis C viral kinetics with the nucleoside polymerase inhibitor mericitabine (RG7128). *Hepatology* **55**:1030–1037.
43. **Chevaliez S, Rodriguez C, Pawlotsky J-M.** 2012. New virologic tools for management of chronic hepatitis B and C. *Gastroenterology* **142**:1303–1313.
44. **Inoue Y, Homma M, Matsuzaki Y, Shibata M, Matsumura T, Ito T, Kohda Y.** Erythrocyte ribavirin concentration for assessing hemoglobin reduction in interferon and ribavirin combination therapy. 2006. *Hepatol. Res.* **34**:23–27.
45. **Dominguez S, Ghosn J, Cassard B, Melica G, Poizot-Martin I, Solas C, Lascaux AS, Bouvier-Alias M, Katlama C, Lévy Y, Peytavin G.** 2012. Erythrocyte and plasma ribavirin concentrations in the assessment of early and sustained virological responses to pegylated interferon-alpha 2a and ribavirin in patients coinfectd with hepatitis C virus and HIV. *J. Antimicrob. Chemother.* **67**:1449–1452.
46. **Breilh D, Djabarouti S, Trimoulet P, Le Bail B, Dupon M, Ragnaud JM, Fleury H, Saux MC, Thiébaud R, Chêne G, Neau D.** 2009. Ribavirin plasma concentration predicts sustained virological response to peginterferon Alfa 2a plus ribavirin in previously treated HCV-HIV-coinfectd patients. *J. Acquir. Immune Defic. Syndr.* **52**:428–430.
47. **Morello J, Rodríguez-Novoa S, Jiménez-Nácher I, Soriano V.** 2008. Usefulness of monitoring ribavirin plasma concentrations to improve treatment response in patients with chronic hepatitis C. *J. Antimicrob. Chemother.* **62**:1174–1180.

Figure legends

Fig. 1: Observed concentrations over time.

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

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

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

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

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

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

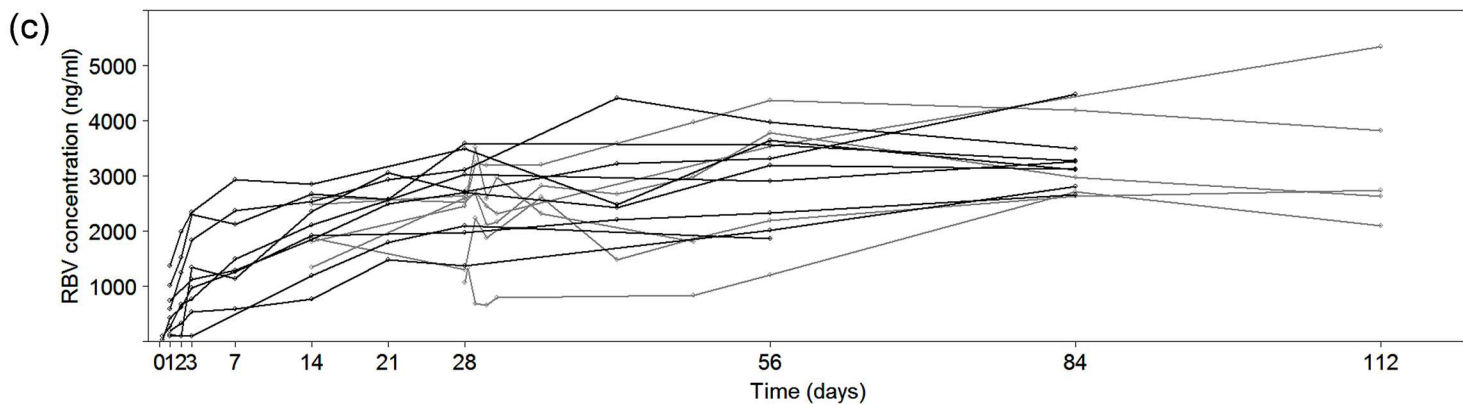
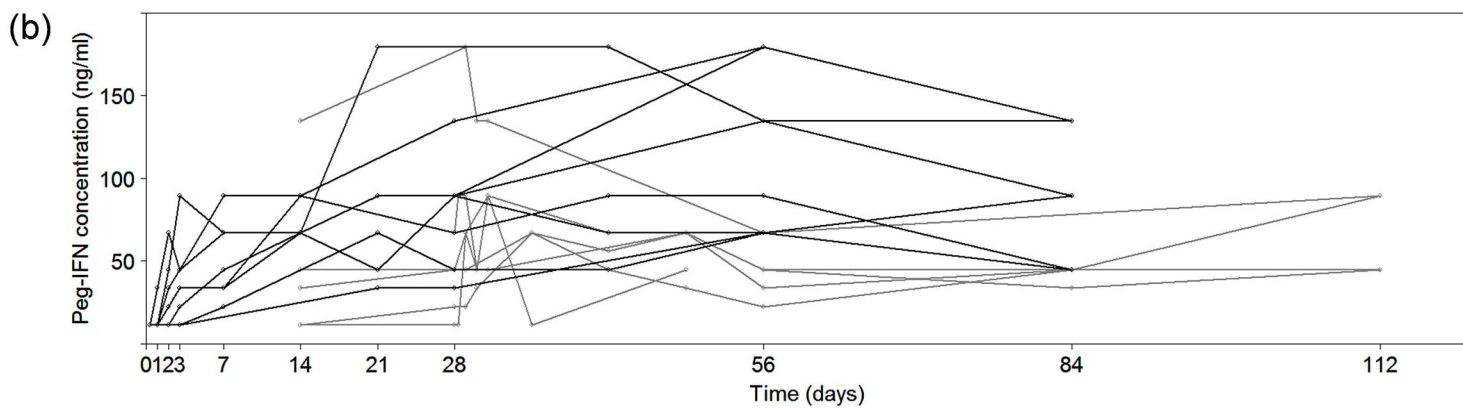
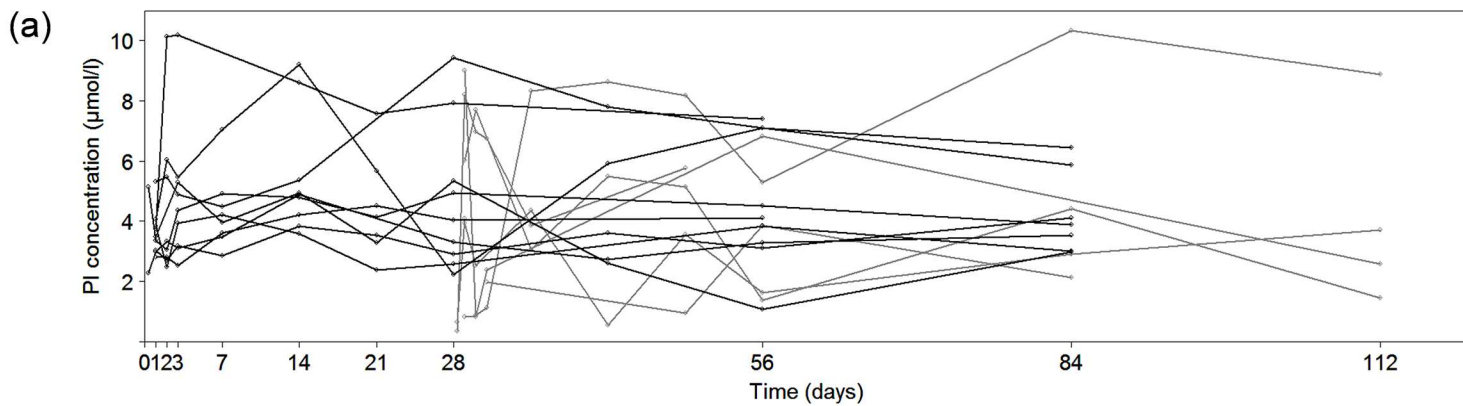
Fig. 4. Relationship between predicted trough concentration at steady state (C_{ss}) and predicted antiviral effectivenesses (ϵ_{ss}).

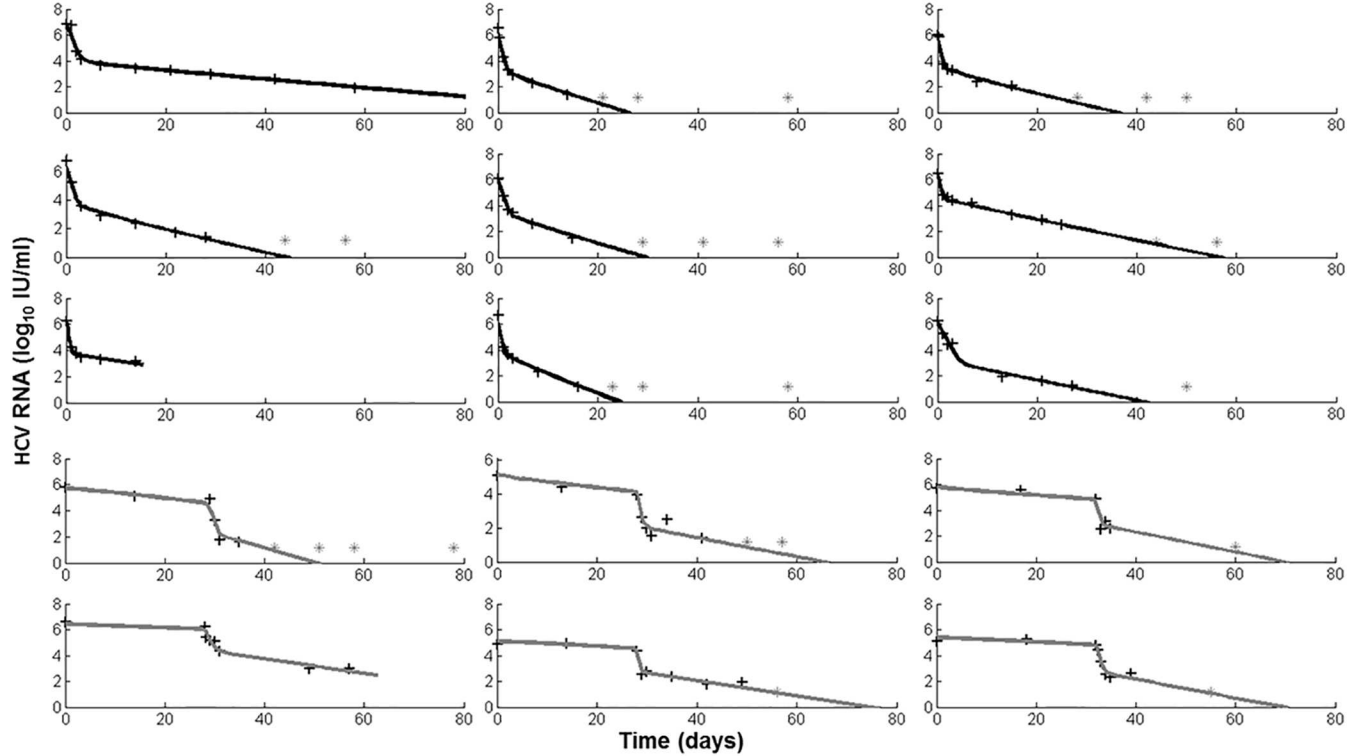
(a) for the protease inhibitor (telaprevir in black and boceprevir in grey, $\mu\text{mol/l}$); (b) for Peg-IFN (Peg-IFN- α 2a in black and Peg-IFN- α 2b in grey, ng/ml). The lines denote the predictions

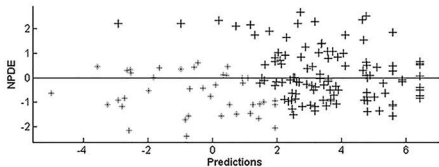
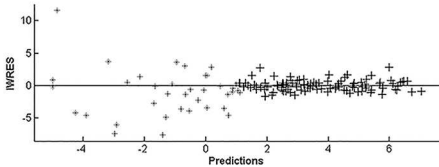
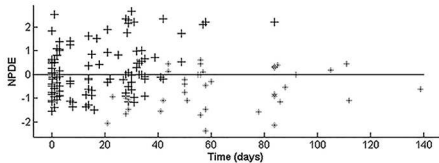
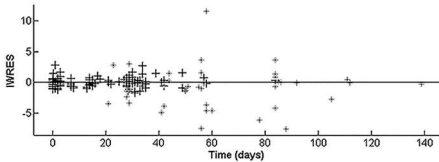
with the mean antiviral effectiveness and the dotted lines denote 95% confidence interval computed with the standard errors predicted by the Fisher Information Matrix.

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

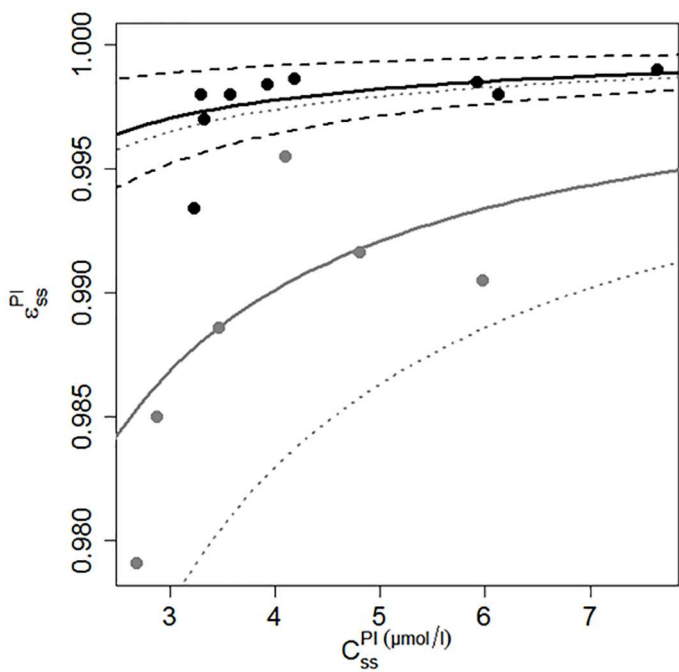
(a) predicted antiviral effectivenesses (ϵ_{ss}) of PIs; (b) predicted antiviral effectivenesses (ϵ_{ss}) of Peg-IFN; (c) δ delta parameter (loss rate of infected cells). P-value from Wilcoxon tests.



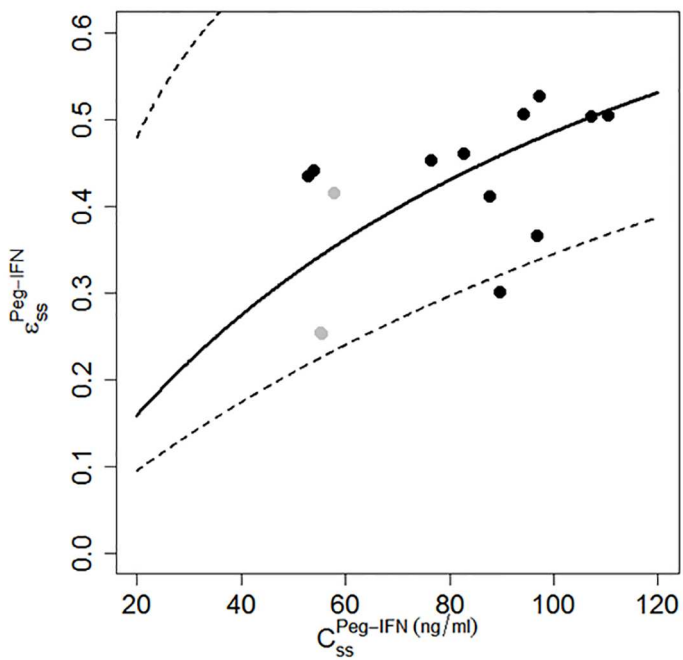


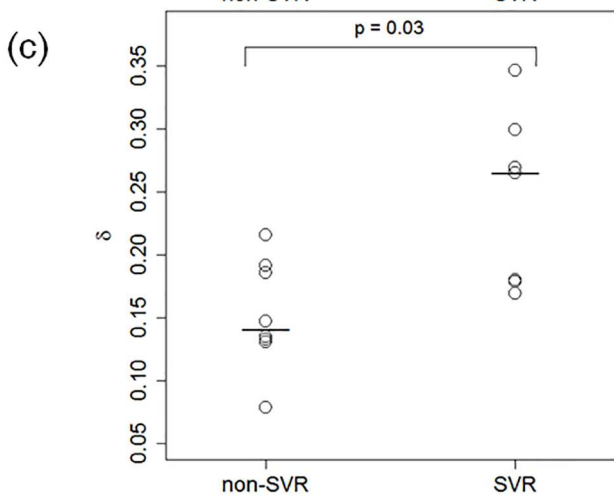
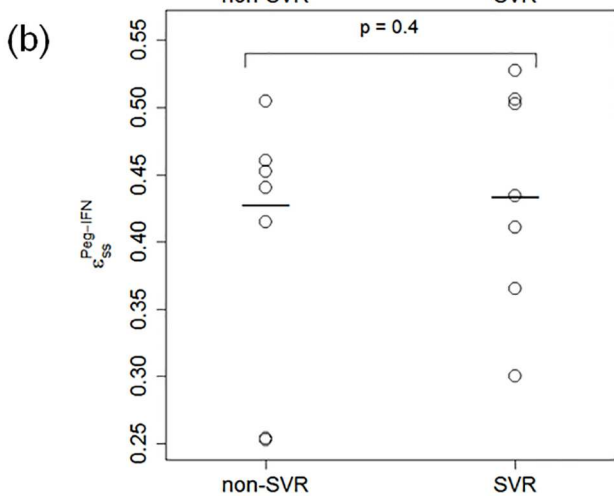
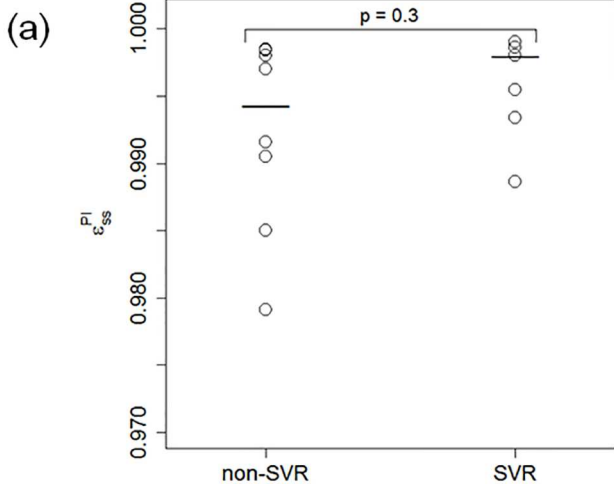


(a)



(b)





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]
$C_{ss}^{\text{telaprevir}}$ (μmol/l)	9	3.77 [2.68; 5.98]
$C_{ss}^{\text{boceprevir}}$ (μmol/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.