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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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)

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

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

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

Results Both PIs achieved similar level of molar concentrations (P=0.5), but there was a significant difference of EC₅₀ (P=0.008), leading to a larger antiviral effectiveness than boceprevir in blocking viral production (99.8% vs 99.0%, respectively, P=0.002). In all patients the antiviral effectiveness of Peg-IFN was modest (43.4%) and there was no significant contribution of RBV exposure on the total antiviral effectiveness. The second phase of viral decline, which is attributed to the loss rate of infected cells, was slow (0.19 day⁻¹) and was higher in patients that subsequently eradicated HCV (P=0.03).

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

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

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

Although these results suggest that a functional cure might be obtained in a large majority of patients, one should keep in mind that issues remain. In particular the proportion of patients with advanced liver disease and cirrhosis and/or who had failed a previous treatment with Peg-IFN/RBV is under represented in the patient population in clinical trials (8–11). The evaluation of the triple therapy in this population was precisely the goal of the ANRS-CO20-CUPIC cohort (Compassionate Use of Protease Inhibitors in viral C Cirrhosis; ClinicalTrials.gov number: NCT01514890) (12), where 511 genotype 1 treatment-experienced cirrhotic patients were included. In this study the SVR rates 12 weeks after treatment discontinuation (SVR12) were equal to 52% and 43% in telaprevir and boceprevir treated patients, respectively (13). The origin of this impaired response might encompass a
variety of factors, in particular impaired drug pharmacokinetics (PK) or limited sensitivity to PI agents and/or Peg-IFN/RBV in this particular population. One way to evaluate treatment antiviral effectiveness and to optimize therapy is to use PK-viral kinetic (VK) models that provide a useful tool to quantitatively describe the relationship between drug exposure and viral response (reviewed in (14)). However no such analysis has been published with boceprevir and results published for telaprevir were mostly based on treatment naive and/or non-cirrhotic patients (15–17).

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

Patients and data

MODCUPIC is a substudy of the French multicentre prospective ANRS-CO20-CUPIC cohort. In four centres, from September 2011 to September 2012, patients chronically monoinfected with HCV genotype 1, compensated cirrhosis (Child-Pugh class A), non-responders to a prior IFN-based therapy and who started triple therapy were recruited. The diagnosis of cirrhosis was made by liver biopsy or non-invasive tests, Fibrotest® or Fibroscan® or Fibrometer® or Hepascore® at the discretion of the investigator, according to the French recommendations (18). The choice between TVR- or BOC-based therapies was at the investigator’s discretion without randomization. TVR-based therapy included 12 weeks of telaprevir (750 mg/8 hours) in combination with Peg-IFN-α2a (180 µg/week) and RBV (1,000 or 1,200 mg/day, depending on body weight) then 36 weeks of Peg-IFN-α2a/RBV (named group telaprevir in the following). BOC-based therapy included 4 weeks (lead-in phase) of Peg-IFN-α2b (1.5 µg/kg/week) or Peg-IFN-α2a (180 µg/week) and RBV (800 or 1,400 mg/day, depending on body weight) then 44 weeks of Peg-IFN-α2b/RBV and boceprevir (800 mg/8 hours) (named group boceprevir in the following). Patients were followed up to six months after treatment discontinuation to assess SVR.

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

Bioanalytical methods

HCV RNA and drug concentrations were measured post PIs initiation at hours 0, 8, days 1, 2, 3 and weeks 1, 2, 3, 4, 8 and 12. Patients treated with boceprevir had two additional VL and 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 µmol/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-α2a and -α2b 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-α2a (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 $C_{\text{Peg-IFN}}(t)$ and $C_{\text{RBV}}(t)$, respectively were fitted using an exponential model to reflect the progressive increase in trough drug concentrations over time:

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

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

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:

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

**Viral kinetic modeling**

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

$$\frac{dI}{dt} = bVT - \delta I \quad \text{Eq. (4)}$$

$$\frac{dV}{dt} = p(1 - \epsilon(t))I - cV$$

where $T$ represent the target cells that can be infected by virus, $V$, with rate $b$. Infected cells, $I$, are lost with rate $\delta$ 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 $T_0 = c\delta/pb$. Treatment is assumed to reduce the average rate of viral production per cell from $p$ to $p(1-\epsilon)$, where $\epsilon$ represents the drug antiviral effectivenesses, i.e., $\epsilon = 0.99$ 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 $E_{\text{max}}$ model assuming a maximum inhibition of 100%:

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

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

Eq. (5)

where $EC_{50}^{\text{PI}}$ (respectively $EC_{50}^{\text{Peg-IFN}}$) is the PI (resp. Peg-IFN) concentration at which the PI (resp. Peg-IFN) is 50% effective, and $C^{\text{PI}}(t)$ (resp. $C^{\text{Peg-IFN}}(t)$) 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:

$$
\left(1 - \varepsilon(t)\right) = \left(1 - \varepsilon^{\text{PI}}(t)\right)\left(1 - \varepsilon^{\text{Peg-IFN}}(t)\right)
$$

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 $E_{\text{max}}$ 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 $V_0$, $c$, $\delta$, $EC_{50}^{PI}$, and $EC_{50}^{Peg-IFN}$ were estimated using non-linear mixed-effect models (NLMEM). In this approach, each individual parameter $\theta_i$ is comprised of a fixed part $\theta$, which represents the mean value of the parameter in the population (fixed effects), and a random part $\eta_i$ chosen from a Gaussian distribution with mean 0 and standard deviation $\omega_i$ that accounts for the inter-individual variability. Therefore, for all parameters $\theta_i = \theta e^{\eta_i}$ where $\eta_i \sim N(0, \omega_i^2)$. Both PK data and Log$_{10}$(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 \((c, \delta, EC_{50}^{Pl})\) 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, \(\varepsilon_{ss}\), of each agent was defined by:

\[
\varepsilon_{ss}^{Pl} = \frac{c_{ss}^{Pl}}{c_{ss}^{Pl} + EC_{50}^{Pl}}
\]

\[
\varepsilon_{ss}^{Peg-IFN} = \frac{c_{ss}^{Peg-IFN}}{c_{ss}^{Peg-IFN} + EC_{50}^{Peg-IFN}}
\]

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-α2a vs -α2b, 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-α2a (8 in telaprevir group and 3 in boceprevir group), 3 patients Peg-IFN-α2b (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] µmol/l i.e. 2,563.0 ng/ml [1,822.0; 4,065.5] and $C_{ss}^{boceprevir} = 3.92$ [3.22; 7.64] µmol/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-α2a and -α2b ($C_{ss}^{Peg-IFN-2a} = 89.6$ [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.
\( V_0^{\text{telaprevir}} = 6.43 \log_{10} \text{IU/ml} \ vs \ V_0^{\text{boceprevir}} = 5.52 \log_{10} \text{IU/ml}, \ P=0.0001 \). 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 \( EC_{50}^{\text{Peg-IFN}} \) 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 \( \log_{10} \) 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 \( EC_{50}^{\text{PI}} \), was significantly higher for telaprevir than boceprevir (\( EC_{50}^{\text{telaprevir}} = 0.009 \ \mu\text{mol/l} \ vs \ EC_{50}^{\text{boceprevir}} = 0.04 \ \mu\text{mol/l}, \ P=0.008 \)). 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 ($\epsilon_{ss}^{telaprevir} = 99.8\% [99.3; 99.9]$ and $\epsilon_{ss}^{boceprevir} = 99.0\% [98.0; 99.6]$, $P=0.002$). 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, $\delta$. We estimated $\delta$ 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, $\delta$, was significantly higher in patients that subsequently achieved SVR (median $\delta^{SVR} = 0.27$ day$^{-1}$ vs median $\delta^{non-SVR} = 0.14$ 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%
(\(\alpha_{EC_{50}^{PI}}\) 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, \(\delta\), about three times smaller than in non-cirrhotic naive-patients (\(\delta\) 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 scarced 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 $\delta$ was higher in patients that subsequently achieved SVR (median $\delta_{SVR} = 0.27$ day$^{-1}$ vs median $\delta_{non-SVR} = 0.14$ day$^{-1}$, P=0.03) suggesting that $\delta$ 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 ($c$, $\delta$, $EC_{50}^{PI}$) was found.
The effect of RBV was analyzed using serum drug concentrations. Some authors preferably used erythrocyte RBV concentration (44), which was not measured in the present study. However a significant relationship was shown between erythrocyte RBV concentrations and serum concentrations (45), suggesting that serum RBV can be used for the assessment of early and sustained virological responses (46, 47).

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


Figure legends

Fig. 1: Observed concentrations over time.
(a) telaprevir in 9 patients (black, µmol/ml) and boceprevir in 6 six patients (grey, µmol/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 (\epsilon_{ss}).
(a) for the protease inhibitor (telaprevir in black and boceprevir in grey, µmol/l); (b) for Peg-IFN (Peg-IFN-α2a in black and Peg-IFN-α2b 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 ($e_{ss}$) of PIs; (b) predicted antiviral effectivenesses ($e_{ss}$) of Peg-IFN; (c) $\delta$ delta parameter (loss rate of infected cells). P-value from Wilcoxon tests.
<table>
<thead>
<tr>
<th>Table 1. Main patient characteristics</th>
<th>Peg-IFN/RBV + telaprevir</th>
<th>Peg-IFN/RBV + boceprevir</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n=9</td>
<td>n=6</td>
<td>n=15</td>
</tr>
<tr>
<td>Age (years), median [min-max]</td>
<td>55 [49-59]</td>
<td>53 [44-64]</td>
<td>55 [44-64]</td>
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<tr>
<td>Males, n (%)</td>
<td>8 (89)</td>
<td>4 (67)</td>
<td>12 (80)</td>
</tr>
<tr>
<td>HCV RNA (log_{10} IU/ml), median [min-max]</td>
<td>6.5 [6.0-6.8]</td>
<td>5.4 [4.9-6.6]</td>
<td>6.2 [4.9-6.8]</td>
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<tr>
<td>HCV genotype, n (%):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>2 (22)</td>
<td>5 (83)</td>
<td>7 (47)</td>
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<tr>
<td>Non 1a</td>
<td>7 (78)</td>
<td>1 (17)</td>
<td>8 (53)</td>
</tr>
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<td>IL28B genotype (rs12979860), n (%):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>2 (22)</td>
<td>-</td>
<td>2 (13)</td>
</tr>
<tr>
<td>C/T</td>
<td>6 (67)</td>
<td>6 (100)</td>
<td>12 (80)</td>
</tr>
<tr>
<td>T/T</td>
<td>1 (11)</td>
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<td>1 (7)</td>
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<tr>
<td>Response to previous bitherapy, n (%):</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Partial responder</td>
<td>-</td>
<td>2 (33)</td>
<td>2 (13)</td>
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<tr>
<td>Null responder</td>
<td>4 (44)</td>
<td>1 (17)</td>
<td>5 (33)</td>
</tr>
<tr>
<td>Relapser</td>
<td>3 (33)</td>
<td>3 (50)</td>
<td>6 (40)</td>
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<tr>
<td>Early discontinuation for adverse event</td>
<td>2 (22)</td>
<td>-</td>
<td>2 (13)</td>
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Table 2. Individual predicted trough concentrations at steady state (C<sub>ss</sub>)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>median [min; max]</th>
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<tbody>
<tr>
<td>C&lt;sub&gt;ss&lt;/sub&gt; telaprevir (µmol/l)</td>
<td>9</td>
<td>3.77 [2.68; 5.98]</td>
</tr>
<tr>
<td>C&lt;sub&gt;ss&lt;/sub&gt; boceprevir (µmol/l)</td>
<td>6</td>
<td>3.92 [3.22; 7.64]</td>
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<tr>
<td>C&lt;sub&gt;ss&lt;/sub&gt; Peg-IFN-α2a (ng/ml)</td>
<td>11</td>
<td>89.6 [52.8; 110.4]</td>
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<tr>
<td>C&lt;sub&gt;ss&lt;/sub&gt; Peg-IFN-α2b (ng/ml)</td>
<td>3</td>
<td>55.4 [55.3; 57.9]</td>
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<tr>
<td>C&lt;sub&gt;ss&lt;/sub&gt; RBV (ng/ml)</td>
<td>15</td>
<td>2,860 [2,428; 3,874]</td>
</tr>
<tr>
<td>Parameter</td>
<td>Estimate</td>
<td>RSE (%)</td>
</tr>
<tr>
<td>-----------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>$V_0^{\text{telaprevir}}$ (log$_{10}$ IU/ml)</td>
<td>6.43</td>
<td>2</td>
</tr>
<tr>
<td>$V_0^{\text{boceprevir}}$ (log$_{10}$ IU/ml)</td>
<td>5.52</td>
<td>3</td>
</tr>
<tr>
<td>$c$ (day$^{-1}$)</td>
<td>3.98</td>
<td>12</td>
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<tr>
<td>$\delta$ (day$^{-1}$)</td>
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<td>$EC_{50}^{\text{Peg-IFN}}$ (ng/ml)</td>
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<tr>
<td>$EC_{50}^{\text{telaprevir}}$ (µmol/l)</td>
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<tr>
<td>$EC_{50}^{\text{boceprevir}}$ (µmol/l)</td>
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<td>$\omega_{V_0}$</td>
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<td>$\omega_c$</td>
<td>0.47</td>
<td>19</td>
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<tr>
<td>$\omega_\delta$</td>
<td>0.42</td>
<td>16</td>
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<tr>
<td>$\omega_{EC_{50}^{\text{Peg-IFN}}}$</td>
<td>0.67</td>
<td>30</td>
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<tr>
<td>$\omega_{EC_{50}^{\text{Pl}}}$</td>
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<td>32</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>0.27</td>
<td>7</td>
</tr>
</tbody>
</table>

$V_0$: baseline viral load; $c$: clearance rate of virus from serum; $\delta$: loss rate of infected cells; $EC_{50}$: half maximal effective concentration; $\omega$: inter-individual variability; $\sigma$: standard deviation of residual error; RSE: relative standard errors of parameter estimates, Pl: protease inhibitor.