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Modeling the interaction between danoprevir and mericitabine in the treatment of chronic HCV infection

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Abstract

Modeling HCV RNA decline kinetics under therapy has proven useful for characterizing treatment effectiveness. Here we model HCV viral kinetics (VK) in 72 patients given a combination of danoprevir, a protease inhibitor and mericitabine, a nucleoside polymerase inhibitor for 14 days in the INFORM-1 trial. A biphasic VK model with time-varying danoprevir and mericitabine effectiveness and Bliss independence for characterizing the interaction between both drugs provided the best fit to the VK data. The average final antiviral effectiveness of the drug combination varied between 0.998 for 100 mg tid of danoprevir and 500 mg bid of mericitabine and 0.9998 for 600 mg bid of danoprevir and 1000 mg bid of mericitabine. Using the individual parameters estimated from the VK data collected over 2 weeks, we were not able to reproduce the low SVR rates obtained in more recent study where patients were treated with a combination of mericitabine and ritonavir-boosted danoprevir for 24 weeks. This suggests that drug-resistant viruses emerge after 2 weeks of treatment and that longer studies are necessary to provide accurate predictions of longer treatment outcomes.

Introduction

Hepatitis C virus (HCV) infection affects over 185 million people worldwide [1]. Antiviral therapy aims to achieve a sustained virologic response (SVR), defined as undetectable plasma levels of viral RNA 24 weeks after cessation of treatment [2]. The current goal of antiviral therapy for HCV is to develop effective combination therapies that are interferon (IFN) free. The first clinical trial of an IFN free drug regimen was the INFORM-1 trial in which a combination of danoprevir, an HCV protease inhibitor, and mericitabine, an HCV polymerase inhibitor, were administered to chronically infected patients for 14 days. This

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combination achieved 5 logs of viral RNA decline without any resistance-associated viral breakthrough [3], thus providing the first proof-of-concept that a combination of different direct acting antivirals (DAAs) without pegylated-IFN or ribavirin can potentially lead to prolonged viral suppression.

In order to optimize combination therapies, it is important to understand how drugs interact as well as the role played by each drug in a combination. Here we use the combination of danoprevir and mericitabine as the basis of a case study. The only other combinations of IFN and ribavirinfree DAAs that have been analyzed were the combination of two HCV polymerase inhibitors, sofosbuvir (SOF) and GS-0938 [4] and the combinations of SOF and ledipasvir (LDV), SOF, LDV and GS-9669, a non-nucleoside polymerase inhibitor and SOF, LDV and GS-9461, an NS3/4A protease inhibitor [3]. For the case of SOF plus GS-0938 [4], the combination of the two drugs was shown to act according to Lowe additivity [5] and the combination was not much more potent than either of the individual drugs. Similarly, when the effectiveness of SOF+LDV was compared to that when either GS-9669 or GS-9461 was added, there was essentially no change in the overall effectiveness, ε, in blocking viral production (For SOF+LDV, ε =0.9998, SOF+LDV+GS-9669, ε =0.9995, and for SOF+LDV +GS-9461, ε =0.9998) [6]. Unlike the cases mentioned above, here we will show that the combination of the protease inhibitor, danoprevir, and the polymerase inhibitor, mericitabine, is much more potent in vivo than the individual drugs and the first-phase viral declines of the two drugs essentially multiplied, i.e., the drugs acted independently and followed what has been called Bliss independence [7].

Materials and Methods

Patients

In INFORM-1, eligible chronically HCV-infected patients were divided into 9 cohorts of 8 patients each and randomly allocated to one of them (Table 1). INFORM-1 cohorts A1, A2, B, C1, C2, D and G, contained treatment-naïve patients. Cohort E was comprised of non-null responders to previous PEG-IFN-α/RBV treatment, defined as patients that had relapsed (i.e., attained an HCV RNA concentration that was undetectable while receiving PEG-IFN/RBV but that became detectable after discontinuation of therapy) or that partially responded with a >2 log10 reduction in viral load at week 12. Cohort F was comprised of null responders to previous PEG-IFN-α/RBV treatment, i.e. patients who achieved <2 log10 reduction in viral load at week 12 or failed to achieve undetectable HCV RNA at the end of treatment. Table 1 shows the different treatment regimens. Among the 72 patients, 55 were infected with genotype 1a, 16 with genotype 1b and one patient was genotype 1 but the subtype was not conclusive. We did not find that the HCV genotype distribution was significantly different between the cohorts (P=0.76, Chi-square test).

Patients in all cohorts were allowed to start PEG-IFN-α/RBV treatment after the end of treatment with the combination of danoprevir and mericitabine [8]. However, we restrict our analysis to the 7 or 14 days of treatment in cohorts A1-A2 and B-G, respectively, which were IFN-free and we excluded patients taking placebo.

HCV RNA levels were measured as previously described [8, 9] from blood samples obtained at 0, 4, 12, 24, 36, 48, 60, 72, 84 hours, on days 4, 5, 6, 9 for all cohorts, and on days 12 and 13 for cohorts B-G. Plasma HCV RNA concentrations were measured with the COBAS TaqMan HCV Test (version 2.0; Roche, Burgess Hill, UK), with a lower limit of quantification of 43 IU/mL and a lower limit of detection of 15 IU/mL [3]. Double-stranded DNA population sequencing of NS5B, NS3/4A, and NS3 protease coding regions was done for all samples at baseline. For groups A1 and A2, drug susceptibility was assessed at baseline (day 1), at the end of monotherapy (day 4), and at the end of combination treatment (day 7) by direct cloning of patient-derived sequences into a shuttle replicon. For all other cohorts, drug susceptibility was tested on days 1 and 7. Patients who had virological rebound (defined as a sustained increase of ≥ 0.5 $\log_{10}$ IU/mL HCV RNA above nadir before the end of treatment) and non-responders (defined as an HCV RNA change from baseline ≤ 0.5 $\log_{10}$ IU/mL) also had population and clonal sequencing of samples at baseline and after rebound. Phenotypic characterization by cloning the patient's NS5B and NS3 protease region into a replicon shuttle vector was also performed to assess drug susceptibility [3].

As previously reported [3], no evidence of treatment-emergent resistance to either compound was identified during the study and 72 of 73 patients in the treatment groups had a continuous decline in viral load, which was maintained throughout dosing. Further, the mericitabine resistance mutation S282T was not detected at baseline, nor was it selected during treatment. Protease inhibitor resistance mutations were detected in 18 patients at baseline [10]. However, no enrichment of minority protease inhibitor resistant variants was observed during treatment and viral population samples were fully susceptible to mericitabine and/or danoprevir suggesting that over the 2 week course of therapy mericitabine suppressed the selection of danoprevir resistance [10]. In vitro studies using the replicon system also showed that mericitabine suppressed development of danoprevir resistance [11]. Because there was a continuous decline in HCV RNA in all but 1 patient, baseline resistant variants did not appear to influence the HCV dynamics in a noticeable way. Based on these findings, we assumed for the purposes of modeling that the viral quasi-species was sensitive to both compounds during the 14 day trial.

We assume patients did not miss any doses as they were confined to a clinical research unit for the duration of the study, with all doses administered by study staff. One patient (#1 in cohort C1) exhibited a viral rebound during therapy ($\geq \log_{10}$ 0.1 increase in HCV RNA/mL at 2 or more consecutive time points). However no evidence of drug resistance could be identified from population sequencing, clonal sequencing or phenotypic analysis during the treatment period [3]. We therefore decided to truncate the data set for this patient using only viral load data before rebound.

Mathematical modeling of viral kinetics under treatment

The HCV RNA kinetics under treatment was modeled using the standard biphasic model [12]

$$\frac{dI}{dt} = \beta TV - \delta I$$
$$\frac{dV}{dt} = (1 - \varepsilon(t))\, pI - cV \quad (1)$$

where T represents target cells, I, infected cells and V, free virus. Since therapy was only given for 7 or 14 days, we assume the target cell level is constant and given by the steady state pre-treatment level of $T_0 = c\delta/\beta p$. Virus, V, infects target cells with rate constant β, generating infected cells, I, which produce new virions at rate p per infected cell. Infected cells are lost at a rate δ per infected cell and virions are assumed to be cleared from circulation at rate c per virion. The effect of danoprevir, mericitabine or their combination on viral production and/or secretion from infected cells is modeled by a factor (1-$\varepsilon(t)$), where $\varepsilon(t)$ is defined as the effectiveness at time t of drug in preventing viral production/secretion.

We used either a constant effectiveness (CE) model [13] for each drug, i.e., $\varepsilon_i(t)$=constant, or a time-varying effectiveness (VE) model, $\varepsilon_i(t)$ not constant, where the subscript i denotes the treatment drug, where i=D danoprevir and i=M for mericitabine For the CE model, the constant was set as:

$$\varepsilon_i = \frac{Dose_i}{ED_{50i} + Dose_i}, \quad (2)$$

where $Dose_i$ is the total amount of a drug i received per day and ED_{50i}, the dose of drug i leading to 50% of the maximal effectiveness for this drug. For the VE model,

$$\varepsilon_i = \frac{Dose_i \left(1 - e^{-k_i t}\right)}{ED_{50i} + Dose_i \left(1 - e^{-k_i t}\right)}, \quad (3)$$

where k_i is a constant characterizing the rate of increase in effectiveness for drug i. This VE model differs from the one that we previously used for danoprevir monotherapy [14] in that the daily drug dose is multiplied by an increasing function of time in both the numerator and denominator of Eq. (3). This model is more physiologically realistic as it supposes that the drug concentration is varying with time rather than just the resulting effectiveness. Also, note that the time to reach z% of the final effectiveness, t_z, is given by the solution of the equation

$$\frac{Dose_i \left(1 - e^{-k_i t_z}\right)}{ED_{50i} + Dose_i \left(1 - e^{-k_i t_z}\right)} = \frac{{}^{z}\!/_{100}\, Dose_i}{ED_{50i} + Dose_i},$$

which a little algebra shows is

$$t_z = -\frac{1}{k_i} ln\left(\frac{\left(1-{}^{z}/_{100}\right)\left(ED_{50i}+Dose_i\right)}{ED_{50i}+\left(1-{}^{z}/_{100}\right)Dose_i}\right). \quad (4)$$

Because previous pharmacokinetic (PK) analyses showed that there were no PK interactions between danoprevir and mericitabine [3], PK effects were not considered in our analyses. Instead, two drug interaction models were tested to identify a possible additive or multiplicative effect of danoprevir and mericitabine. Rearranging Eq. (2), one finds that $\frac{\varepsilon_i}{1-\varepsilon_i}=\frac{Dose_i}{ED_{50i}}$. Assuming that the doses of the two drugs weighted by their respective ED_{50}'s simply add, then the total effectiveness, ε, is given by the Loewe additivity model defined as

$$\varepsilon=\frac{\frac{\varepsilon_D}{1-\varepsilon_D}+\frac{\varepsilon_M}{1-\varepsilon_M}}{1+\frac{\varepsilon_D}{1-\varepsilon_D}+\frac{\varepsilon_M}{1-\varepsilon_M}} \quad (5)$$

Alternatively, if the effect of the two drugs is multiplicative, i.e. if

$$1-\varepsilon=\left(1-\varepsilon_D\right)\left(1-\varepsilon_M\right), \quad (6)$$

then one obtains the Bliss independence model [5, 7], where ε is the effectiveness resulting from the interaction between danoprevir and mericitabine, and ε_D and ε_M are the danoprevir and mericitabine effectiveness, respectively, seen in monotherapy studies. Because the terms $(1-\varepsilon_D)$ and $(1-\varepsilon_M)$ multiply, this is also sometimes called a multiplicative model. Notice that if we rearrange Eq. (5) we find that for Lowe additivity

$$1-\varepsilon=\frac{1}{1+\frac{\varepsilon_D}{1-\varepsilon_D}+\frac{\varepsilon_M}{1-\varepsilon_M}}=\frac{\left(1-\varepsilon_D\right)\left(1-\varepsilon_M\right)}{\left(1-\varepsilon_D\right)\left(1-\varepsilon_M\right)+\varepsilon_D\left(1-\varepsilon_M\right)+\varepsilon_M\left(1-\varepsilon_D\right)}, \quad (7)$$

which is always smaller than $(1-\varepsilon)$ for the Bliss independence model.

Parameter estimation and statistical methods

We simultaneously fit the data of all patients from all cohorts. Population parameter estimates and inter-individual variability (IIV) estimates were obtained using a maximum-likelihood method implemented in MONOLIX version 4.2 (http://software.monolix.org). In MONOLIX, the estimation algorithm include a simulation of the left-censored data (below the limit of quantification (BLQ) data) with a right-truncated Gaussian distribution to handle BLQ data. Further details about mixed effect models and the population approach used here, as well as other details about parameter estimation are given in the Supplementary Information.

Model selection

We fit the model given by Eq. (1), with the effectiveness of mericitabine and danoprevir given by the CE or VE model, and combined according to Loewe additivity, Eq. (2), or Bliss independence, Eq. (3), to the data from all the cohorts simultaneously using a population approach. Parameters for each individual subject were estimated as empirical Bayes estimates (Supplementary information).

We then compared the fits of the different models using the Bayesian information criterion (BIC) [15], as well as goodness-of-fits plots. We also considered how precise the parameter estimates were by analyzing their relative standard error (RSE).

We tested the effect of genotype and treatment status on the different parameters with an ascending procedure. We started from the model without covariates and then added each of these possible covariates. The covariate was kept in the model if it improved the BIC and if its effect was statistically different from 0, i.e. not null, using a Wald test. We then tested to add each covariate with a similar method.

Evaluation of interaction between danoprevir and mericitabine

To determine the best model for the interaction between danoprevir and mericitabine, for each drug we compared the estimated ED_{50} for the individual subjects in the groups getting combination therapy with the ED_{50} for individual subjects estimated when the drug is taken alone during the first three days in cohorts A1 and A2.

SVR predictions

To compare our results to the INFORM-SVR study, we used our model to predict the SVR rate after 24 weeks of treatment with 100 mg bid of danoprevir and 1000 mg bid of mericitabine, the doses used in INFORM-SVR [16]. We used the individual viral kinetic parameters estimated here from the 72 INFORM-1 patients given these drug doses and then computed the proportion of 72 subjects whose viral load reached the cure boundary of 3×10^{-5} IU/mL [17] after 24 weeks of treatment.

Results

The patterns of viral decline for cohorts A1 and A2 depended on the drug that was used in monotherapy during the first 4 days. If treatment was initiated with mericitabine alone (cohort A1), the viral decline during the first 4 days was slow and the total viral load decline reached 0.46 $\log_{10}$IU/mL. It was then followed by a rapid decline when danoprevir was added (Fig. 1). Conversely, in cohort A2, where treatment was initiated with danoprevir alone, a rapid decline was observed during the first 4 days that reached 1.87 $\log_{10}$IU/mL. Adding mericitabine induced a smaller drop in the viral load. The viral decline at day 7 was similar in both cohorts (3.02 $\log_{10}$IU/mL).

Viral load decline during the combination of danoprevir and mericitabine was biphasic followed by a rebound in one patient (Fig. 2). We only examined the kinetics during the decline (see Methods). The average viral load declines at day 14 are presented in Table 1.

Viral kinetics with the combination of danoprevir and mericitabine

We first compared the various models describing HCV VK under the combination of danoprevir and mericitabine, using the CE and VE models for each drug and Loewe additivity or Bliss independence models to characterize the interaction between the two drugs. We found that the model with varying effectiveness for danoprevir and mericitabine and Bliss independence between them provided the best estimates, fits and BIC (see Table S1). Not surprisingly, as danoprevir and mericitabine act at different places in the viral life cycle, we found that Bliss independence provided a lower BIC, i.e., was a better model, than the additive interaction irrespective of whether the CE or VE model was used for danoprevir and mericitabine. We therefore only present the results using Bliss independence hereafter.

We found that the patient treatment status (i.e. treatment naïve, non-null responder or null responder) was significantly associated with the estimated free virus clearance rate (P=0.0003). However, HCV genotype was not significantly associated with the estimated ED_{50} of mericitabine, ED_{50M}, or danoprevir, ED_{50D}, or with the transition rates to the final effectiveness of mericitabine and danoprevir, k_M, and k_D, respectively (Table 2).

We estimated all model parameters (Table 2) and found that the model fit the data well (Fig. S1). The initial viral load was estimated as V_0= 6.36 log IU/mL across all the cohorts, the infected cell death/loss rate, δ=0.29 d^{-1}, and the viral clearance rate, c, varied between 7.54 d^{-1} and 9.73 d^{-1} depending on the patient treatment status (Table 2). The ED_{50} for danoprevir was estimated as ED_{50D}=2.09 mg and the transition rate to danoprevir maximal effectiveness as k_D=0.73 d^{-1}, which leads to maximal danoprevir effectiveness estimates of 0.993, 0.997, 0.998 and 0.999 and times of 1.9 hrs, 1.0 hrs, 0.48 hrs and 0. 24 hrs to reach 90% of the maximal effectiveness for 100 mg tid, 200 mg tid, 600 mg bid and 900 mg bid, respectively (Table 3). The times to reach 99% of the maximal effectiveness are about ten times longer (Table 3).

The ED_{50} for mericitabine was estimated as ED_{50M} =533 mg and the transition rate to mericitabine maximal effectiveness was k_M=0.42 d^{-1}, which leads to maximal mericitabine effectiveness estimates of 0.652 and 0.789 for 500 mg bid and 1000 mg bid (Table 3), and times to reach 90% maximal effectiveness of 3.4 and 2.5 days, respectively and times to reach 99% effectiveness of 8.4 and 7.2 days, respectively. That mericitabine may take a long time to reach its final effectiveness was previously reported [18].

The average final effectiveness for the combination of danoprevir and mericitabine varied from 0.998 in patients receiving 100 mg tid of danoprevir and 500 mg bid of mericitabine to 0.9998 in patients receiving 900 mg bid of danoprevir and 1000 mg bid of mericitabine. Table 3 shows the predicted final effectiveness for danoprevir, mericitabine and their combination, while Figs. 3 and S2 show how the effectiveness of the individual drugs and their combination change in time for all cohorts.

We next examined whether our model and estimated parameters could be used to predict the outcome of longer term therapy. In the INFORM-SVR trial HCV genotype 1 patients were given 100 mg bid of danoprevir and 1000 mg bid of mericitabine plus or minus ribavirin (RBV) for 12 or 24 weeks [16]. In the arm without RBV there were unacceptably high

relapse rates and at 12 weeks these patients were also given PEG-IFN/RBV. SVR rates were thus only available in the RBV containing arm. Using our model, we simulated the outcome of 24 weeks on 100 mg bid of danoprevir and 1000 mg bid of mericitabine using parameters estimated for the 72 patients from INFORM-1 who received this drug combination, ignoring the influence of RBV. Nonetheless, we predict that after 24 weeks only 4 patients would not reach the cure boundary (see Methods). This led to a predicted SVR rate of 94.4%, which is significantly higher than the SVR rate of 37.9% found in INFORM-SVR study [16].

Discussion

Mathematical modeling has provided significant insights into the viral kinetics observed during PEG-IFN/RBV based therapy for HCV [12, 19]. Recently, viral kinetic models have also been used for characterizing treatment with DAAs including the protease inhibitors telaprevir and danoprevir [14, 17, 20], the NS5A inhibitor daclatasvir [21], the nucleoside polymerase NS5B inhibitors mericitabine [18], sofosobuvir and GS-0938 [4], as well as combinations of sofosobuvir and ledipasvir, sofosobuvir, ledipasvir and GS-9669 and sofosobuvir, ledipasvir and GS-9461 [6]. A model incorporating interactions between sofosbuvir and GS-0938, two nucleotide polymerase inhibitors, concluded that the drugs acted additively [4].

In this work we assessed the interaction between two DAAs belonging to different drug classes, namely danoprevir, a protease inhibitor, and mericitabine, a polymerase inhibitor. The models used to describe each drug's effectiveness reflect the current knowledge about their mode of action. The VE model for danoprevir is consistent with the rapid increase of danoprevir effectiveness observed in a previous monotherapy trial [14]. The VE model for mericitabine, with a slower transition rate to the maximal effectiveness, reflects the slower increase of its effectiveness [18], most likely due to the several phosphorylations necessary to activate the drug intracellularly.

The free virus clearance rate, c, was significantly associated with patient prior treatment status and was lower for treatment naïve patients (7.56 d^{-1}) compared to treatment experienced patients (8.00 d^{-1} (p=0.47) and 9.77 d^{-1} (p=0.0012) in non-null responders and null responders, respectively), with no significant difference between the treatment-naïve and non-null responders. Alternatively, the higher estimate of c in the null responder cohort may reflect the fact that pharmacokinetic analyses revealed that this cohort had substantially higher danoprevir exposure than previously reported for treatment naïve patients [3]. Further, Rong et al. [22] showed that danoprevir in addition to inhibiting HCV replication also had activity in inhibiting viral assembly/secretion. If this activity was higher in the null responder group due to greater danoprevir exposure, then a higher estimate of c would be expected as their would be less residual viral secretion to slow viral clearance in the null responder cohort [21].

Our estimate of δ obtained during combination therapy is higher than that estimated previously during monotherapy with danoprevir (0.184 d^{-1}) [14] or mericitabine (0.029 d^{-1} in null responders) [18]. Population-based sequence analysis of patients under danoprevir monotherapy experiencing virologic plateau (low δ) indicated the presence of resistant virus

at the end of treatment (day 14) with decreased susceptibility to danoprevir [23]. In the presence of combination therapy, viruses resistant to one drug can still respond to the other drug in the combination, and may explain the higher δ estimated in the present study.

For telaprevir, both in monotherapy and in combination with PEG-IFN, a positive correlation between δ and the $\log_{10}$ transformed final treatment effectiveness was found [17, 24]. Similar to the case of danoprevir monotherapy [14], no such correlation could be established for the combination of danoprevir and mericitabine (data not shown)

A number of different IFN-free combination therapies are being studied in clinical trials [6, 25-29]. The INFORM-1 study showed that the combination of a protease inhibitor, danoprevir and a nucleoside polymerase inhibitor, mericitabine, can successfully decrease HCV viral load by nearly 5 logs over a period of 14 days, with no resistance-associated viral breakthrough [3]. Here, via a drug interaction analysis we showed that the two drugs act independently. This leads to an increased final effectiveness compared to monotherapy with either of these two drugs. A multiscale model taking into consideration the replication, export and degradation of intracellular HCV RNA was recently introduced [21] and applied to the analysis of viral kinetics in patients treated with danoprevir monotherapy [22]. However, this model was developed for a short study (2 days) and assumes constant drug effectiveness and rates of RNA production and degradation and might not be valid for longer studies as it is the case for INFORM-1.

Whereas the INFORM-1 study showed that a combination of two different DAAs, danoprevir and mericitabine, can successfully decrease HCV viral load by nearly 5 logs over a period of 14 days, with no viral breakthrough, in INFORM-SVR study, patients treated with a combination of RBV and 1000 mg of mericitabine and 100 mg ritonavir-boosted danoprevir twice daily for 24 weeks led to poor SVR rates [30]. Using the patient parameters estimated here, we predicted that the SVR rate for this combination would be 94% if drug resistance did not emerge. However, as 1000 mg mericitabine bid takes 7.2 days to reach 99% of its maximum effectiveness, the patients in INFORM-SVR were effectively exposed to danoprevir monotherapy early in treatment. Thus, not unexpectedly danoprevir resistance mutations were detected in 96/97 patients who failed to achieve SVR and for whom population sequencing data was available. Interestingly, in the shorter 14 day INFORM-1 trial, no evidence of emergent drug resistance could be identified. Thus, the discrepancy between our predicted SVR rate and the outcome of INFORM-SVR could be associated with the emergence of drug-resistance virus after 14 days of treatment which could not be captured by the short-term data we analyzed. Other factors associated with our model assumptions, such as ignoring baseline protease inhibitor resistant variants [10] and our assumption of constant target cells, could also have led to differences between our predictions and the INFORM-SVR outcomes. In fact, in standard viral dynamic models with constant target cells both wild-type and drug resistant variant level decline under therapy [31] and hence they are not suitable for long-term predictions in situations where drug resistance plays a role. This suggests that longer studies including on treatment measurements of the frequencies of drug resistant variants and the use of models describing both target cell and drug-resistant virus dynamics are necessary to make accurate SVR

predictions with this drug combination and suggests the same may be true for other DAA combinations in which one or more drugs has a low barrier to resistance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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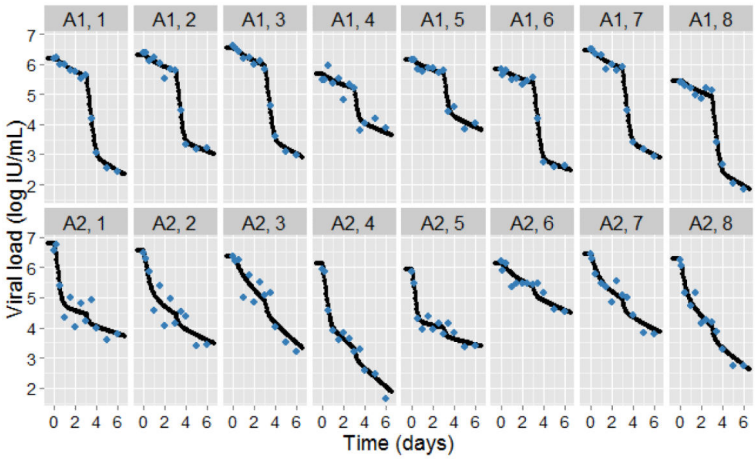

Figure 1.

The viral kinetics of individual patients based on predictions of the interaction model during 3 days of monotherapy with mericitabine (A1) or danoprevir (A2) followed by 4 days of combined therapy with both drugs. The best-fit prediction of viral RNA decline is shown by the black curve, and the measured HCV RNA is shown by blue dots.

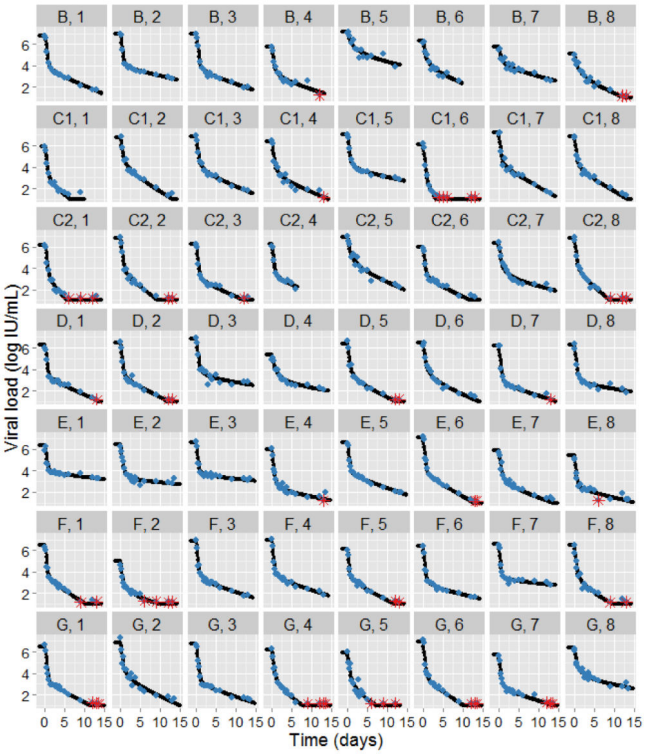

Figure 2.

The viral kinetics of individual patients based on predictions of the Bliss independence interaction model during 14 days of combined danoprevir and mericitabine therapy. The best-fit prediction of viral RNA decline is shown by the black curve, and the measured HCV RNA is shown by blue dots. Red stars represent data below the limit of quantification. Data were not collected at days 9, 10 and 12 for patient #4 in cohort C2. Data were truncated for patient #1 in cohort C1.

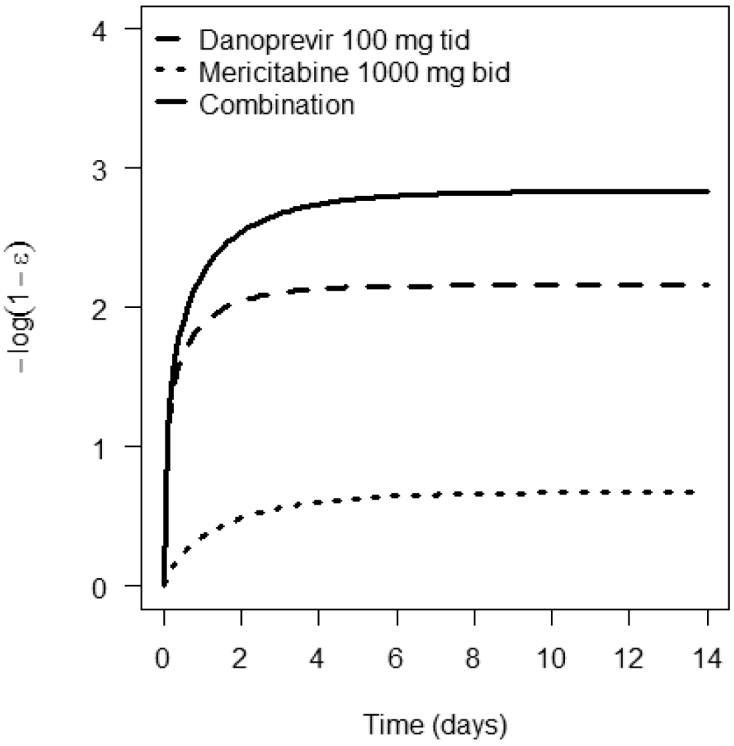

Figure 3.
Predicted effectiveness of danoprevir monotherapy 100 mg tid (dashed lines), mericitabine monotherapy 1000 mg bid (dotted lines) and their combination (solid line) for patients infected with genotype 1A HCV virus in cohort D.

Table 1

Baseline characteristics of the patients

Cohort	Danoprevir dose (regimen)	Mericitabine dose (regimen)	Patient type	Genotype			Initial viral load Log_{10} IU/mL	Viral load decline at day 14 Log_{10} IU/mL
				1a	1b	NA		
A1	100 mg (tid) D4-7	500 mg (bid) D1-7	Treatment Naïve (TN)	6	1	1	6.06	-
A2	100 mg (tid) D1-7	500 mg (bid) D4-7	TN	6	2	0	6.26	-
B	100 mg (tid) D1-14	500 mg (bid) D1-14	TN	6	2	0	6.27	3.9
C1	200 mg (tid) D1-14	500 mg (bid) D1-14	TN	7	1	0	6.64	5.0
C2	100 mg (tid) D1-14	1000 mg (bid) D1-14	TN	5	3	0	6.44	4.9
D	200 mg (tid) D1-14	1000 mg (bid) D1-14	TN	6	2	0	6.25	4.6
E	600 mg (bid) D1-14	1000 mg (bid) D1-14	Non-null responder	7	1	0	6.18	4.0
F	900 mg (bid) D1-14	1000 mg (bid) D1-14	Null responder	7	1	0	6.31	4.7
G	900 mg (bid) D1-14	1000 mg (bid) D1-14	TN	5	3	0	6.46	4.9

NA: genotype 1, but the subtype was not conclusive. Non-null responders to previous PEG-IFN-α/RBV treatment, i.e. if they had relapsed (an HCV RNA concentration that was undetectable while receiving standard of care but that became detectable after discontinuation of therapy) or if they partially responded with a >2 log10 reduction in viral load at week 12. Null responders to previous PEG-IFN-α/RBV treatment, i.e. patients who achieved <2 log10 reduction in viral load at week 12 or failed to achieve undetectable HCV RNA at the end of treatment.

Table 2

Population parameter estimates for combination therapy with danoprevir (D) and mericitabine (M)

Parameter		Population parameter (RSE %)	IIV % (RSE %)
V_0 (log IU/mL)		6.36 (14)	115 (9)
c (d^{-1})	Naïve	7.54 (4)	9 (53)
	Null	7.94 (7)	
	Non-null	9.73 (7)	
δ (d^{-1})		0.29 (10)	65 (9)
EC_{50D} (mg)		2.09 (23)	106 (15)
k_D (d^{-1})		0.73 (36)	159 (16)
EC_{50M} (mg)		533 (36)	50 (59)
k_M (d^{-1})		0.415 (52)	-

RSE: Relative Standard Error; IIV: Inter-Individual Variability

Table 3

Average final effectiveness for danoprevir monotherapy, mericitabine monotherapy and the combination of danoprevir and mericitabine

Cohort	Danoprevir dose (regimen)	Mericitabine dose (regimen)	Danoprevir final effectiveness (time to 90%, 99% final effectiveness)	Mericitabine final effectiveness (time to 90%, 99% final effectiveness)	Combination final effectiveness
A1	100 mg (tid) D4-7	500 mg (bid) D1-7	0.993 (1.9 hrs, 17.0 hrs)	0.652 (3.4 days, 8.4 days)	0.998
A2	100 mg (tid) D1-7	500 mg (bid) D4-7	0.993 (1.9 hrs, 17.0 hrs)	0.652 (3.4 days, 8.4 days)	0.998
B	100 mg (tid) D1-14	500 mg (bid) D1-14	0.993 (1.9 hrs, 17.0 hrs)	0.652 (3.4 days, 8.4 days)	0.998
C1	200 mg (tid) D1-14	500 mg (bid) D1-14	0.997 (1.0 hrs, 9.6 hrs)	0.652 (3.4 days, 8.4 days)	0.999
C2	100 mg (tid) D1-14	1000 mg (bid) D1-14	0.993 (1.9 hrs, 17.0 hrs)	0.789 (2.5 days, 7.2 days)	0.999
D	200 mg (tid) D1-14	1000 mg (bid) D1-14	0.997 (1.0 hrs, 9.6 hrs)	0.789 (2.5 days, 7.2 days)	0.999
E	600 mg (bid) D1-14	1000 mg (bid) D1-14	0.998 (0.48 hrs, 5.0 hrs)	0.789 (2.5 days, 7.2 days)	0.9996
F	900 mg (bid) D1-14	1000 mg (bid) D1-14	0.999 (0.24 hrs, 3.4 hrs)	0.789 (2.5 days, 7.2 days)	0.9998
G	900 mg (bid) D1-14	1000 mg (bid) D1-14	0.999 (0. 24 hrs, 3.4hrs)	0.789 (2.5 days, 7.2 days)	0.9998