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Telaprevir-based Treatment Effects on Hepatitis C Virus in Liver and Blood

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Abstract

Background—Understanding hepatitis C virus (HCV) replication has been limited by access to serial samples of liver, the primary site of viral replication. Our understanding of how HCV replicates and develops drug resistant variants in the liver is limited.

Methods—We studied 15 patients chronically infected with genotype 1 HCV treated with telaprevir (TVR)/pegylated-interferon alfa/ribavirin. Hepatic fine needle aspiration was performed pretreatment and at hour 10, days 4 and 15, and week 8 after initiation of antiviral therapy. We measured viral kinetics, resistance patterns, TVR concentrations, and host transcription profiles.

Results—All patients completed all protocol defined procedures that were generally well tolerated. First phase HCV decline (baseline-treatment day 4) was significantly slower in liver than in plasma (slope plasma, -0.29 ; liver, -0.009 [$p < 0.001$]) while second phase decline (post-treatment day 4 to 15) did not differ between the two body compartments (-0.11 and -0.15 , respectively, $p = 0.1$). TVR-resistant variants were first detected in the plasma, but not in the liver (where only wild-type virus was detected). Based upon NS3 sequence analysis, no compartmentalization of viral populations was observed between plasma and liver compartments. Gene expression profiling revealed strong tissue-specific expression signatures. Human intrahepatic TVR concentration, measured for the first time, was lower compared to plasma on a gram per milliliter basis. We found moderate heterogeneity between HCV RNA levels from different intrahepatic sites, indicating differences in hepatic microenvironments.

Conclusion—These data support an integrated model for HCV replication wherein the host hepatic milieu and innate immunity control the level of viral replication, and the early antiviral

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response observed in the plasma is predominantly driven by inhibition of hepatic high-level HCV replication sites.

Keywords

HCV; liver sampling; pharmacokinetics; telaprevir; biomarkers; resistance transcript profiling

Introduction

Chronic hepatitis C virus (HCV) infection affects ~120 million people worldwide and nearly 5 million people in the United States (1, 2). In other viral infections such as HIV, a high viral replication rate is often associated with a cytopathic effect, limiting viral replication by killing host cells in combination with a strong immune response, thereby limiting viral replication and spread. In contrast, HCV can persist for decades in a human host, despite a high replication rate of 10^{12} virions per day (3). Chronic HCV infection can result in chronic inflammation that promotes ongoing liver damage (4). The addition of HCV protease inhibitors, telaprevir (TVR; T) and boceprevir, to pegylated-interferon/ribavirin (P/R) have been shown to enhance efficacy and may shorten treatment duration (5, 6). These agents act by blocking polyprotein processing and formation of the HCV replication complex, thereby inhibiting viral replication.

Difficulty in intrahepatic sampling has been a limitation in the understanding of HCV biology. Kinetic studies in patients receiving interferon monotherapy or P/R revealed a biphasic decline in plasma HCV RNA levels, wherein the first phase corresponds to clearance of viral particles from circulating blood and the second phase corresponds to the decay of viral replication in infected hepatocytes or hepatocyte loss (3, 7). More recent studies in patients receiving TVR monotherapy or T/P/R described a biphasic decline wherein both the first and second phase declines were more rapid than that obtained with P/R (8, 9). Prior studies during P/R treatment suggested that baseline intrahepatic interferon stimulated gene (ISG) expression levels are increased and that the degree of induction is predictive of treatment efficacy suggesting that liver transcript analysis may identify predictors of response (10, 11).

Although a few studies have employed core needle biopsy (CNB) for liver sampling in HCV infection (11, 12), serial measurements using CNB are difficult. An alternative is liver fine needle aspiration (FNA), which has significantly reduced morbidity compared to standard liver CNB (13). We performed serial liver FNAs at baseline and during antiviral therapy on 15 patients with genotype 1 chronic HCV infection, 9 treatment-naïve, and 6 P/R prior non-responders (NR) to understand the correlates of intrahepatic and peripheral viral kinetics. Specifically, we assessed: i) kinetics of wild-type and TVR-resistant variants, ii) pharmacokinetics of TVR, and iii) host gene expression. While the mechanisms of action and efficacy of the treatment regimens are well understood; within the liver the *in vivo* exposure of TVR, the degree of inhibition of viral replication, and the rate of clearance of HCV RNA from infected cells are unknown.

Methods

Fifteen genotype 1 chronic HCV-infected patients aged 18–65 years received TVR 750 mg q8h, pegylated-interferon alfa-2a 180 µg weekly, and weight-based ribavirin (1000 or 1200 mg/day) (T/P/R) for 12 weeks followed by at least 12 additional weeks of P/R. Nine patients were treatment-naïve (<4 weeks of prior P/R treatment), and six patients were prior non-responders to P/R. All patients had stage 1–3 fibrosis, with 11 patients with stage less or equal 2. Written informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Weill-Cornell Institutional Review Board.

Patients were hospitalized overnight for treatment initiation. Liver FNAs were performed at baseline (within 7 days before treatment initiation), at 10 hours post-initiation of triple therapy and on days 4 and 15, and after a protocol amendment, at week 8 (Supplementary Figure 1).

Plasma HCV RNA levels were measured using the COBAS® AmpliPrep / COBAS® TaqMan® HCV Test, version 1 test with Ampliprep extraction Roche Diagnostics, Indianapolis, IN) with a quantification limit of 43 IU/ml and a detection limit of 7 IU/ml for genotype 1.

Statistical Analysis

Statistical analysis was performed using SAS (SAS Institute Inc., Cary, NC, USA) and R (<http://www.r-project.org/>). Continuous variables are presented by their means or medians and their standard deviations (SD) or inter-quartile ranges (IQR). Categorical variables are summarized using counts and percentages. HCV RNA viral kinetics in plasma and liver were modeled through non-linear mixed effects models adjusted for left censoring of the HCV RNA levels. TVR concentrations in plasma and liver over time were compared through a mixed effects model. Repeatability of the FNA procedure for assessment of intrahepatic HCV RNA levels was evaluated through the coefficient of individual agreement. Comparison between continuous variables, if not explicitly stated, was conducted through Wilcoxon's signed rank or rank sum tests, and between categorical variables, through Fisher's exact test. More detailed information on the statistical methods can be found in the supplementary material.

The following methods are described in the supplementary materials: (i) RNA isolation from liver and determination of HCV RNA copy number; (ii) HCV population and clonal sequencing in liver and plasma; (iii) gene expression assessment using the nCounter® Analysis System (NanoString Technologies, Seattle, WA); (iv) TVR measurements; and (v) statistical methods.

Results

Patients

A total of 15 patients received at least one dose of T/P/R. Eleven (73%) patients were Caucasian, 3 (20%) African-American, and 1 (7%), Hispanic. Median age was 55

(interquartile range: 46.5, 58) years and 9 (60%) patients were male (Supplementary Table 2). Eleven patients had HCV genotype 1a, 2 were IL28B genotype CC and 2, genotype TT. All patients completed all protocol-defined FNAs, and the procedure was generally well tolerated; no pain was reported by eight of fifteen patients during or after any of the FNA procedures (Supplementary Material).

Treatment course and outcome

A total of 9 (60%) patients achieved a sustained virological response (SVR) and 6 did not achieve SVR after T/P/R. Among these later patients, 4 experienced viral breakthrough, 1 was lost to follow up at week 8, and 1 self-discontinued on day 15 due to side effects. Among treatment-naïve patients, 7/9 (77.8%) achieved SVR, while among prior NRs to P/R, 2/6 achieved SVR. Among genotype 1a patients, 7/11 achieved SVR and among 1b patients, 2/4 achieved SVR. Both IL28B CC genotype patients achieved SVR (7/13 for non-CC).

Drug concentration

The overall TVR concentrations differed significantly in plasma compared to liver ($p=0.007$) (Figure 1a) with median (interquartile range) liver to plasma ratios of 0.72 (0.4, 1.01), 0.47 (0.33, 0.78), 0.57 (0.34, 0.76), 0.61 (0.42, 0.84) at each of the four time points when concentrations were assessed. TVR concentrations in human plasma and liver are 5.5-to-14.4 fold and 2.3-to-14.9 fold higher, respectively, than the IC_{50} in the HCV replicon (14) and 0.7-to-6.3 fold and 2.3-to-6.1 fold, respectively, above the IC_{90} (15).

HCV RNA decline in plasma and liver

In plasma, the mean log₁₀-transformed copies/ml HCV RNA levels were 6.5±0.8 at pre-dose, 4.9±0.9 at 10 hours, and 3.2±0.6 on treatment day 4 (Figure 1B, Supplementary Figure 2). In liver, HCV RNA levels were quantified relative to the total mass of RNA and the mean log₁₀-transformed copies/ng total HCV RNA levels were 4.2±1.2 pre-dose, 4.0±1.3 at 10 hours, and 3.9±1.2 at 4 days after treatment initiation.

Between baseline and day 4, we observed a slower decrease in hepatic HCV RNA relative to plasma (median log₁₀-transformed HCV RNA decline slope plasma, 0.29; liver, 0.009 [$p<0.0001$]). Between treatment days 4 and 15, median rates of HCV RNA decline in liver and plasma were not significantly different (0.11 and 0.15, respectively, $p=0.104$). All post-treatment week 8 intrahepatic HCV RNA levels were undetectable and a higher slope of HCV RNA decrease was observed between day 15 and week 8 in liver (−0.04) than in plasma (−0.01; $p=0.02$), potentially attributable to low plasma HCV RNA levels.

Modeling HCV RNA in plasma and liver

A detailed description of the statistical modeling is given in Supplementary material. In plasma, HCV RNA levels were modeled according to Neumann *et al.* 1998 (3), assuming constant effectiveness, accounting for left censoring and adjusting the infected hepatocyte loss rate for patient's sex, treatment-naïve status and baseline ALT levels (Table 1a, Supplementary Figure 3). Infected hepatocyte loss rate (d), which characterizes the second phase of viral decline (3), was positively correlated with the baseline ALT level, male

gender, and treatment-naïve status. Addition of the parameter δ as determined from the liver model (below) did not improve the plasma model fit.

The plasma model did not fit the viral kinetics observed in the liver well (based on AICc; Supplemental Table 5). As such, the model was modified by setting the virion clearance rate equal to the infected hepatocyte loss rate and setting the treatment efficacy of reducing new infections to 1. Both baseline ALT and AST levels were positively correlated with viral decline ($\rho = 0.69$, $p = 0.006$ and $\rho = 0.70$, $p = 0.008$, respectively). We observed high correlation ($\rho = 0.93$, 95% confidence interval 0.78, 0.98) between ALT and AST levels. Model parameters that corresponded to the best fit are shown in Table 1b (also Supplementary Figure 3). The association between viral decline and ALT tended to differ between patients depending upon whether they were infected with HCV subtypes 1a or 1b (Table 1b). No association was observed between inflammation (portal and lobular) and viral decline. We estimated individual values for δ for each patient as determined by plasma and liver models, and the median values (0.58, IQR (0.35, 0.80) vs. 0.44 IQR (0.40, 0.57)) differed significantly between compartments ($p = 0.03$) (Table 1c).

Assessment of intrahepatic HCV RNA variability

We assessed the repeatability of the FNA sampling technique based on 33 pairs of liver HCV RNA samples from 12 of the 15 study patients (Figure 2A). None of the patients had more than 1 pair of liver HCV RNA samples per time point. In 27% (5 of 18) of pairs with detectable values, we observed a ratio of the larger value to the lower of >3 . Twelve pairs had undetectable HCV RNA in one of the FNA passes, of them 5 had an HCV RNA value <190 copies/ng. The paired differences in $\log_{10}(\text{HCV RNA})$ measurements are plotted versus their average (Figure 2B). In general, we observed moderate agreement (based on the coefficient of agreement [CIA]) between HCV RNA measurements in liver obtained from the two separate FNA passes (Supplementary material including suppl. Table 6), with CIA ranging between 0.41 and 0.59 at the different time points. The 95% CI for the ratio between the two samples was (0.003; 906.8-fold).

Viral resistance to TVR

We sequenced wild-type (WT) and TVR-resistant variants in all samples with quantifiable HCV RNA using population sequence analysis and, in a subset of samples ($n=7$), by clonal analysis (Table 2). Sequencing was successful in 100% (15/15) of baseline plasma and in 80% (12/15) of baseline liver samples. The plasma subtype distribution, as determined by the NS3 sequencing method, was 73% ($n=11$) 1a and 27% ($n=4$) 1b.

Prior to treatment, only WT virus was detected in both plasma and liver in all patients. In contrast, during or after the treatment phase, TVR-resistant variants (16, 17) were observed in 7 patients (2 SVRs and 5 non-SVRs) in plasma (V36M/A, T54A/S, R155K/G, A156T/V, R155M+A156A/T, V36M+R155K) (Supplementary Table 7, Figure 3) whereas only WT virus was detected in liver, even for those visits where resistant variants were observed in the plasma (Tables 2 and Supplementary Table 8).

Phylogenetics of liver and plasma sampled virus

Liver and plasma viral populations were compared for 12 baseline samples and 16 on-treatment visits using population sequence analysis. Highly sensitive clonal sequence analysis, able to detect minor viral populations (< 5%), was performed on samples from three patients. A total of 14 isolates (median 73 [range 63–89] and 77 [range 59–94] clones) were sequenced from each plasma and liver FNA isolate, respectively. These patients were selected because they had the largest number of genomic differences between liver and plasma populations based on population sequence results within the NS3-4A proteins.

The phylogeny generated from the population sequence analysis suggested no compartmentalization between liver and plasma. All isolates from each patient clustered together to the exclusion of any isolates from another patient, regardless of the timing of sampling (minimum bootstrap support: 93%) (Figure 4). Significantly, this result was supported by clonal analysis indicating lack of clustering between liver and plasma isolates from 3 patients sampled at 2 or more timepoints (Figure 5). These data suggest that both liver and plasma isolates sampled the same underlying viral populations.

Gene expression between compartments—As expected, we observed highly significant differences between liver and blood samples. At baseline, samples could be differentiated based on tissue type rather than donor origin (*i.e.*, blood and liver samples from a patient never clustered together, but rather clustered with the samples of the same tissue (Figure 6A). Differentiation between tissue types was observed in 203/267 loci assessed ($p < 0.05$) with 154 of these loci having significantly different expression levels after Bonferroni correction (Supplementary Table 7; p -value range: $1.2e-17$, $1.6e-4$).

We also assessed degree of induction of these loci by T/P/R on Day 1 relative to baseline in blood and liver between SVR and non-SVR patients. After type I error correction, no loci achieved statistical significance for prediction of treatment outcome. However, we observed a trend towards statistical significance between SVR and non-SVR patients for intrahepatic induction levels of 35 loci ($p < 0.05$). Furthermore, 8 of 12 loci previously identified as predictive of P/R-based treatment outcome (18, 19) were significantly higher in SVR patients ($p < 0.05$ prior to type 1 error correction) (Figure 6B).

Discussion

In this study, we evaluated four distinct aspects of HCV infection and treatment (viral decay, drug exposure, HCV genomic variation, and transcriptional profiling) to compare liver and blood before and after initiation of antiviral therapy. We observed the following. 1) The initial decline in intrahepatic HCV RNA lagged compared to plasma while the second phase decline was similar. 2) TVR-resistant variants were detected in the plasma, but not in the liver (where only WT virus was detected). 3) No compartmentalization in liver and plasma viral populations was observed based upon NS3 sequence analysis. 4) As expected, gene expression profiling revealed strong tissue-specific expression signatures in baseline and on-treatment samples. However, loci previously described as associated with SVR in P/R-treated patients were not predictive of treatment outcome in T/P/R-treated patients in this study with a small sample size that included a large subset of prior P/R non-responders. 5)

Human intrahepatic TVR concentration, measured for the first time, was lower compared to plasma on a mass per volume basis.

The relationship between HCV RNA decline in human liver and blood has not been previously assessed largely due to the difficulty of serially sampling the liver using conventional CNB. In plasma, our modeling results (virion clearance rate $c=16.5$ and mean infected hepatocyte loss rate $\delta=0.61$) were comparable to other studies ($c=13.4$ and $\delta=.58$ (9), $c=15.93$ and $\delta=0.29-0.62$ (8)) in T/P/R-treated patients. Unlike prior studies, we adjusted our model for the left censoring in the data. We performed repeated FNA sampling in liver, and in comparison to plasma, we observed a significantly slower decrease in HCV RNA during the first phase of viral decline. While the results we obtained regarding virus decline in plasma during the first phase were consistent with previously published reports, in liver there was very little change in HCV RNA during this time interval. Previously published models of viral decline in plasma (3, 9) fit our plasma data well, but not the liver data. In order to optimize the fit of the liver model, we set the viral clearance rate (c) as equal to the loss rate of productively infected cells (δ) and the efficacy of treatment in reducing new infections equal to 1. Consequently, as indicated by the significantly slower rate of intrahepatic viral decline when compared to blood expressed by the loss of HCV cellular replication and/or the death rate of infected cells, δ , we conclude that HCV RNA decay is markedly delayed in liver compared to plasma.

To compare the hepatic and peripheral viral populations, we performed population and clonal sequence analysis using the NS3 region because its gene product, the HCV protease, is the TVR target. Although this protein is highly conserved relative to many other HCV proteins, NS3 sequence analysis was sufficiently sensitive to demonstrate separate phylogenies for each patient's viral quasispecies. Overall, we did not observe any substantial differences in NS3 hepatic or peripheral viral population sequences sampled from a single patient at the same time point. Interestingly, we observed TVR-resistant variants exclusively in blood during the first phase of viral decline. These variants have been shown to be "uncovered" when replication of the much larger population of WT virus is inhibited (16, 20); their presence per se was not associated with treatment failure. Alternatively, as NS3 inhibition has recently been shown to interfere a multitude of viral processes including assembly and release (21), replication complexes that contain protease resistance mutations might preferentially be exported to the plasma compartment.

These findings are consistent with TVR's primary role in inhibition of WT and lower-level TVR-resistance variants (defined as a 3- to 25-fold increase in IC_{50} compared to WT) conferred by V36A/M, T54A/S, R155K/T, and A156S HCV protease variants (16). In patients who did not achieve an SVR with treatment, the resistant population becomes predominant in plasma. Since mutation is a clonal process and only a very limited sample of liver is evaluated with FNA, it is not surprising that virus detected in liver is WT, at the same time that the product of the mutant clones is detected in plasma.

Taken together, these results might be explained by at least two models: 1) "Delayed Interferon Clearance" or 2) "High and Low Level Sites of HCV Replication". In model #1, between baseline and post-treatment day 4, TVR inhibits replication of the majority of WT

and low-level TVR-resistant variants, but interferon-induced clearance of infected cells has not yet commenced. TVR-induced inhibition of replication of these variants is permissive for replication of primarily higher-level TVR-resistant variants, which were observed on day 4. The absence of resistant variants in liver at the same time point suggests that these resistant variants may be a minority population of the total infection compared to the majority non-replicating WT virus that has not yet been cleared by the host's immune response. Alternatively, they may be absent within the microenvironment sampled. In summary, HCV RNA concentration and sequencing data together support a model in which TVR rapidly and efficiently inhibits viral replication in hepatocytes, but host immunity is required to clear infected cells from the liver, as observed during the second phase of viral decline during P/R treatment.

In model #2, we posit that intrahepatic HCV replication is not uniform, *i.e.*, a few sites of high-level replication are surrounded by many sites of low-level replication. *In vitro* studies have described limited spread of infection, ~0.5% of Huh7 cells with HCV JFH1 virus infection (22). *In vivo* studies have illustrated foci of HCV-infected cells in liver samples from patients with chronic HCV infection (23). Furthermore, other studies (24) showed that HCV genomic RNA was variably distributed throughout HCV-infected livers and that HCV replicative-intermediate RNA are distributed in a focal pattern. Thus, the majority of plasma HCV RNA pretreatment may have originated from high-level replication sites. Because the NS3 sequence from plasma and liver show no compartmentalization, the host is likely the major determinant of the level of viral replication. HCV replication has been shown to be higher in cultured cells where part of the innate immune response is knocked out (25). Additionally, hepatocyte production of CXCR3-associated chemokines, the primary promoter of intrahepatic inflammation, is distributed focally throughout the hepatic parenchyma (26). With initiation of antiviral therapy, we hypothesize that inhibition of WT virus in the high level replication sites contributes to the bulk of the first phase decline. With treatment continuation, the lower level replication sites will decline due to prolonged T/P/R suppression in combination with host immunity. Resistant variants are created through the error-prone HCV replication machinery and are present at a higher level in foci with high replication levels. Furthermore, these sites are the primary contribution to the plasma quasispecies from which resistant variants arise and are "uncovered" when WT and low-level resistant variants are inhibited by T/P/R.

Hepatic TVR concentrations were lower relative to plasma and significantly different than preclinical studies in rats or dogs where liver concentrations were 10- to 20- or 1- to 3-fold higher, respectively, relative to plasma (14, 27). Although liver-to-plasma ratios for some protease inhibitors measured *in vivo* in preclinical species have been shown to have reasonable correlation to the human situation (27), our data suggest that TVR preclinical studies in animals may not entirely reflect the *in vivo* human situation. TVR has not been found to be a substrate for uptake transporters (17), although it is a substrate for, and an inhibitor of, P-glycoprotein (28).

In contrast to earlier studies performed in P/R-treated patients (11, 12, 18, 19), we did not identify an ISG signature in plasma or blood that was predictive of treatment outcome. These findings may result from differences in therapeutic regimen (P/R versus T/P/R), or

patient characteristics. Additionally, the degree of hepatic ISG induction under P/R treatment may be a more significant driver of SVR probability than it is in a treatment regimen that includes a direct-acting antiviral.

To test the hypothesis that the liver is uniformly infected with HCV and that different microenvironments mount comparable immune responses during infection, we compared HCV RNA and host transcriptional profiling across multiple FNA isolates in the same patient. Overall, HCV RNA levels were moderately consistent between two FNA isolates sampled at a single time point: for the majority of FNA samples, HCV RNA measurements were highly correlated. Measurement variability could result from differential cell-type composition within the sample, different stages of fibrosis between samples, or differences in the microenvironment surrounding the area sampled that could result in differences in the infected cell rate or in the intrinsic viral replication rate within that microenvironment. Interestingly, we also noted intrahepatic variability in gene expression profiles as indicated by poor agreement between loci measured on two separate FNA samples. While this analysis was retrospective and different analytical methods were used to measure each isolate, it crudely compares transcriptional levels across isolates; the results suggest variability in expression of loci within the liver. Taken together, these results suggest existence of marked differences within liver microenvironments in terms of the quantity of HCV present and host transcriptional activity.

In this study, we employed the FNA technique in a novel manner, serial hepatic sampling over a short interval during antiviral treatment. While potential limitations of FNA sampling includes variable quantities of liver and cross-contamination by plasma, expression signatures on liver samples and the vast majority of sampled loci were differentially expressed between samples from the two compartments. Small sample volumes obtained by FNA may also have impacted our ability to detect resistant variants in the liver. Due to the difficulty obtaining samples by aspiration, patients with cirrhosis were excluded, although advanced fibrosis could have affected the HCV RNA decline rates or intrahepatic TVR concentration. These results suggest that liver comprised the major portion of the FNA sample with minimal plasma contamination. Additionally, FNA TVR concentrations in the liver were lower than in plasma, supporting minimal cross-contamination. Overall, our results support an integrated model for HCV replication where i) HCV in the plasma are the progeny of HCV replicating in the host liver. ii) Different hepatic microenvironments support a few high-level and a larger number of low-level sites of viral replication. iii) When patients are treated with a potent antiviral drug, the first phase rapid viral decline observed in the plasma is predominantly driven by the inhibition of WT HCV at high-level sites of viral replication in the liver. iv) Drug-resistant variants, generated by the error-prone HCV replication site, are also more likely to originate from sites of high-level replication. For anti-HCV therapy to be efficacious, it must inhibit the virus at all sites of viral replication in the liver. The first phase decline of plasma HCV precedes the clearance of virus in the liver. The second slope of virus decline in the plasma parallels that in the liver and is a better predictor of the overall viral clearance rate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations

HCV	hepatitis C virus
TVR or T	telaprevir
P/R	pegylated-interferon/ribavirin
telaprevir	T/P/R, pegylated-interferon and ribavirin
ISG	interferon stimulated gene
CNB	core needle biopsy
FNA	fine needle aspiration
NR	nonresponder
SVR	sustained virological response
IQR	interquartile range
CIA	coefficient of individual agreement
ALT	alanine aminotransferase
WT	wild-type
IC	inhibitory concentration

References

1. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis.* 2005; 5:558–567. [PubMed: 16122679]
2. Chak E, Talal AH, Sherman KE, Schiff ER, Saab S. Hepatitis C virus infection in USA: an estimate of true prevalence. *Liver Int.* 2011; 31:1090–1101. [PubMed: 21745274]

3. Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, Perelson AS. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon- α therapy. *Science*. 1998; 282:103–107. [PubMed: 9756471]
4. Zeremski M, Petrovic LM, Talal AH. The role of chemokines as inflammatory mediators in chronic hepatitis C virus infection. *J Viral Hepat*. 2007; 14:675–687. [PubMed: 17875002]
5. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med*. 2011; 364:2405–2416. [PubMed: 21696307]
6. Poordad F, McCone J Jr, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, et al. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med*. 2011; 364:1195–1206. [PubMed: 21449783]
7. Zeuzem S, Herrmann E, Lee JH, Fricke J, Neumann AU, Modi M, Colucci G, et al. Viral kinetics in patients with chronic hepatitis C treated with standard or peginterferon α 2a. *Gastroenterology*. 2001; 120:1438–1447. [PubMed: 11313314]
8. Adiwijaya BS, Kieffer TL, Henshaw J, Eisenhauer K, Kimko H, Alam JJ, Kauffman RS, et al. A viral dynamic model for treatment regimens with direct-acting antivirals for chronic hepatitis C infection. *PLoS Comput Biol*. 2012; 8:e1002339. [PubMed: 22241977]
9. Guedj J, Perelson AS. Second-phase hepatitis C virus RNA decline during telaprevir-based therapy increases with drug effectiveness: implications for treatment duration. *Hepatology*. 2011; 53:1801–1808. [PubMed: 21384401]
10. Feld JJ, Nanda S, Huang Y, Chen W, Cam M, Pusek SN, Schweigler LM, et al. Hepatic gene expression during treatment with peginterferon and ribavirin: Identifying molecular pathways for treatment response. *Hepatology*. 2007; 46:1548–1563. [PubMed: 17929300]
11. Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, Heim MH. Interferon signaling and treatment outcome in chronic hepatitis C. *Proc Natl Acad Sci U S A*. 2008; 105:7034–7039. [PubMed: 18467494]
12. Lau DT, Fish PM, Sinha M, Owen DM, Lemon SM, Gale M Jr. Interferon regulatory factor-3 activation, hepatic interferon-stimulated gene expression, and immune cell infiltration in hepatitis C virus patients. *Hepatology*. 2008; 47:799–809. [PubMed: 18203148]
13. Chhieng DC. Fine needle aspiration biopsy of liver - an update. *World J Surg Oncol*. 2004; 2:5. [PubMed: 15025788]
14. Perni RB, Almquist SJ, Byrn RA, Chandorkar G, Chaturvedi PR, Courtney LF, Decker CJ, et al. Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. *Antimicrob Agents Chemother*. 2006; 50:899–909. [PubMed: 16495249]
15. Lin K, Perni RB, Kwong AD, Lin C. VX-950, a novel hepatitis C virus (HCV) NS3-4A protease inhibitor, exhibits potent antiviral activities in HCV replicon cells. *Antimicrob Agents Chemother*. 2006; 50:1813–1822. [PubMed: 16641454]
16. Kieffer TL, De Meyer S, Bartels DJ, Sullivan JC, Zhang EZ, Tigges A, Dierynck I, et al. Hepatitis C viral evolution in genotype 1 treatment-naïve and treatment-experienced patients receiving telaprevir-based therapy in clinical trials. *PLoS One*. 2012; 7:e34372. [PubMed: 22511937]
17. Vertex Pharmaceuticals. [Accessed January 13, 2013] Prescribing information for incivik. Available at: http://pi.vrtx.com/files/uspi_telaprevir.pdf
18. Chen L, Borozan I, Sun J, Guindi M, Fischer S, Feld J, Anand N, et al. Cell-type specific gene expression signature in liver underlies response to interferon therapy in chronic hepatitis C infection. *Gastroenterology*. 2010; 138:1123–1133. e1121–1123. [PubMed: 19900446]
19. Dill MT, Duong FH, Vogt JE, Bibert S, Bochud PY, Terracciano L, Papassotiropoulos A, et al. Interferon-induced gene expression is a stronger predictor of treatment response than IL28B genotype in patients with hepatitis C. *Gastroenterology*. 2011; 140:1021–1031. [PubMed: 21111740]
20. Kieffer TL, Sarrazin C, Miller JS, Welker MW, Forestier N, Reesink HW, Kwong AD, et al. Telaprevir and pegylated interferon- α -2a inhibit wild-type and resistant genotype 1 hepatitis C virus replication in patients. *Hepatology*. 2007; 46:631–639. [PubMed: 17680654]

21. McGivern D, Feng Z, Lovell W, Hamlett C, Saalau-Bethell S, Graham B. Dissecting Activities of an Allosteric NS3 Inhibitor Using Cell-based Assays [abst# 102]. HEP DART 2013: Frontiers in Drug Development for Viral Hepatitis. 2013 December 8–12 2013; Big Island, HI. Global Antiviral Journal. 2013; 9(Suppl2):89.
22. Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med. 2005; 11:791–796. [PubMed: 15951748]
23. Liang Y, Shilagard T, Xiao SY, Snyder N, Lau D, Cicalese L, Weiss H, et al. Visualizing hepatitis C virus infections in human liver by two-photon microscopy. Gastroenterology. 2009; 137:1448–1458. [PubMed: 19632233]
24. Chang M, Marquardt AP, Wood BL, Williams O, Cotler SJ, Taylor SL, Carithers RL Jr, et al. In situ distribution of hepatitis C virus replicative-intermediate RNA in hepatic tissue and its correlation with liver disease. J Virol. 2000; 74:944–955. [PubMed: 10623757]
25. Zhong J, Gastaminza P, Cheng G, Kapadia S, Kato T, Burton DR, Wieland SF, et al. Robust hepatitis C virus infection in vitro. Proc Natl Acad Sci U S A. 2005; 102:9294–9299. [PubMed: 15939869]
26. Zeremski M, Petrovic LM, Chiriboga L, Brown QB, Yee HT, Kinkhabwala M, Jacobson IM, et al. Intrahepatic levels of CXCR3-associated chemokines correlate with liver inflammation and fibrosis in chronic hepatitis C. Hepatology. 2008; 48:1440–1450. [PubMed: 18798334]
27. Reddy MB, Morcos PN, Le Pogam S, Ou Y, Frank K, Lave T, Smith P. Pharmacokinetic/Pharmacodynamic predictors of clinical potency for hepatitis C virus nonnucleoside polymerase and protease inhibitors. Antimicrob Agents Chemother. 2012; 56:3144–3156. [PubMed: 22470110]
28. Garg V, van Heeswijk R, Lee JE, Alves K, Nadkarni P, Luo X. Effect of telaprevir on the pharmacokinetics of cyclosporine and tacrolimus. Hepatology. 2011; 54:20–27. [PubMed: 21618566]

Figure 1.

A) Plot of the mean (standard deviation) ln-transformed A) telaprevir (TVR) concentration in plasma and liver at each time point. B) Plot of the mean (standard deviations) $\log_{10}$ -transformed HCV RNA levels over time. Undetectable levels in liver and plasma are depicted on the lower limit of detection line (LLOD). The vertical dashed line corresponds to TVR, pegylated-interferon, RBV (T/P/R) treatment initiation. Pre-dose corresponds to baseline before treatment initiation; H6-hour 6 after treatment initiation; H10-hour 10, D4-day 4, D8-day 8, D15-day 15, W4-week 4, W8-week 8, W12-week 12. At each time point, the extensions depict the respective standard deviations.

Figure 2.

A) Plot of 33 log₁₀-transformed HCV RNA paired samples from liver obtained through two fine needle aspiration (FNA) passes on 12 patients. Dashed lines refer to 'values below level of detection, imputed as half the level of detection. B) Bland and Altman plot of the paired differences in the two liver log₁₀-transformed HCV RNA samples versus their average. The horizontal solid line corresponds to the mean difference, while the horizontal dashed lines correspond to the lower and upper 95% limits of agreement (LoA). The plotted symbol is the patient's identification number.

Figure 3. Population sequence of plasma (NS3•4A) and liver fine needle aspiration (FNA) (NS3) of patients who did not obtain a sustained virological response. See figure legend for symbol explanation.

Figure 4.

(GT 1a_Figure 4a; GT 1b_Figure 4b): Unrooted phylogenetic trees of population sequence of the NS3 region of plasma (black) and liver fine needle aspiration (FNA) (red) isolates, for both genotype 1a (A) and 1b (B). The sample identification number (ID) of each patient is indicated, as is the time at which the sample was collected (*D-7*, 1 week before treatment initiation; *D1* 10h after the first treatment; *D4*, Day 4). To assess method variability, a subset of isolates was processed in replicate, with the parenthetical number after the sample ID. Monophyly of virus from each patient is well supported (minimum bootstrap support of 93%). In no instance did the tree support monophyly of virus from either compartment. Distance scale: scale which represents the number of differences between sequences (e.g. 0.1 means 10% differences between two sequences)

Figure 5.

Phylogenetic analysis of clonal sequence data. Clones derived from liver and plasma isolates are identified by filled triangles and squares, respectively. Where multiple amplifications were performed (to avoid amplification artifacts), they are indicated with the color code. For reference, the population sequence analysis of each isolate was also included in the phylogeny and is depicted in the open symbols. Each panel is drawn to its own scale, with the scale bar indicating the number of substitutions per nucleotide position.

Figure 6.

A) Robust baseline differential expression between tissue types. The heatmap displays the intensity of the signal of each gene (arranged in columns) and indicated in the color legend along a relative scale, with each sample represented by a single row. B) Eight loci previously implicated in the literature (see manuscript) as being associated with responsiveness during pegylated interferon-ribavirin treatment tended to show differences between sustained virological responders (SVR) and those who did not obtain an SVR (NR).

Table 1a

Results from the model fit for HCV RNA in plasma, assuming constant effectiveness and adjusting the infected hepatocyte loss rate, δ , for patients' sex (β_{Male}), treatment naïve status ($\beta_{\text{Tr.naive}}$) and alanine aminotransferase (ALT) levels (β_{ALT}) as determined at screening. Parameter ε_p is the efficacy of treatment in blocking virion production, c is the rate per virion at which virions are cleared, and t_0 is the time at which the drug becomes effective.

Parameters Estimates				
Parameter	Estimate	Standard Error	Pr > t	Lower Upper
ε_p	0.99	0.002	<.001	0.989 0.998
c	16.50	3.27	<.001	9.44 23.57
δ_0	0.12	0.08	0.12	-0.038 0.286
t_0	0.18	0.02	<.001	0.13 0.22
β_{ALT}	0.004	0.001	0.02	0.0007 0.0065
$\beta_{\text{Tr.naive}}$	0.16	0.07	0.0494	0.0005 0.32
β_{Male}	0.31	0.07	<.001	0.17 0.45

Results from the model fit for HCV RNA in liver (assuming model 3- as indicated in Supplementary Table 5) and adjusting the loss rate δ for patients' $\ln(\text{ALT})$ level measured at screening (β_{ALT}), sub-genotype 1a ($\beta_{1\text{A}}$) and the interaction between $\ln(\text{ALT})$ and sub-genotype 1a ($\beta_{1\text{A}*\text{ALT}}$). Parameter P is the rate per infected hepatocyte at which virions are produced. Abbreviation: ALT, alanine aminotransferase.

Table 1b

Parameter Estimates					
Parameter	Estimate	Standard Error	Pr > t	Lower	Upper
δ_0	-6.78	3.07	0.046	-13.41	-0.15
P	12.40	10.07	0.24	-9.36	34.16
β_{ALT}	2.04	0.89	0.04	0.12	3.97
$\beta_{1\text{A}}$	6.36	3.10	0.06	-0.33	13.05
$\beta_{1\text{A}*\text{ALT}}$	-1.81	0.89	0.06	-3.74	0.12

Table 1c

Estimated values of parameter δ for each patient from plasma and from liver HCV RNA. For patient with PID 1007, δ from liver was not estimated since all HCV RNA liver measurements were undetectable. δ is the rate per cell at which infected hepatocytes are lost.

Patient	δ from plasma	Standard Error of δ from plasma	δ from liver	Standard Error of δ from liver
1001	0.80	0.10	0.57	0.11
1002	0.91	0.14	0.60	0.10
1003	0.50	0.06	0.34	0.09
1004	0.33	0.06	0.44	0.11
1006	0.30	0.06	0.42	0.08
1007	0.37	0.12		
1008	0.88	0.17	0.78	0.14
1009	0.59	0.09	0.43	0.09
1010	0.35	0.06	0.44	0.08
1011	0.95	0.12	2.61	1.03
1012	0.84	0.10	0.59	0.09
1013	0.69	0.10	0.40	0.08
1014	0.58	0.08	0.54	0.10
1015	0.29	0.06	0.27	0.09
1016	0.74	0.10	0.27	0.10

Table 2

Population Sequence (NS3 region) of Paired Plasma and Liver Samples (5 discordant pairs and 23 concordant pairs). D-7, Baseline (predose); D1 6H, Day 1 6 hours post dose; D1 10H, Day 1 10 hours post dose; D4, Day 4; nd, not done; na, not available; WT, wild type; LLOQ, lower limit of quantitation; TVR, telaprevir; IU, international units.

	Patient	Genotype/S subtype	Timepoint	Plasma		Liver			
				HCV RNA (IU/mL)	TVR Resistant Variants Observed	Primary Liver Sample	TVR Resistant Variants Observed	HCV RNA (copies/total RNA)	TVR Resistant Variants Observed
Discordant Pairs	1006	1A	D4	1,109	T54A, A156T, R155K	WT	WT	33,308	<LLOQ
	1010	1A	D4	1,374	V36A, R155K/G	WT	WT	3,371	<LLOQ
	1011	1B	D1 10H	141,519	T54T/A	WT	WT	2,466	ND
	1014	1A	D4	2,018	V36V/M	WT	WT	92,901	53,842
	1015	1B	D4	3,662	A156A/T	WT	WT	73,763	ND
Concordant Pairs	1002	1A	D-7	3,050,727	WT	WT	WT	18,025	24,118
			D1, 6H	60,054	WT	WT	NA	ND	NA
	1003	1A	D-7	4,081,131	WT	WT	WT	18,427	18,069
			D1 10H	549,890	WT	WT	WT	17,204	29,213
			D-7	3,878,672	WT	WT	WT	30,271	18,169
	1004	1A	D1 10H	172,565	WT	WT	WT	75,929	ND
			D4	5,111	WT	WT	WT	75,336	60,953
	1006	1A	D-7	1,365,871	WT	WT	WT	63,457	<LLOQ
	1008	1A	D-7	645,930	WT	WT	WT	91,886	<LLOQ
			D1 10H	6,112	WT	WT	WT	19,028	ND
	1009	1A	D-7	246,830	WT	WT	WT	46,950	152,915
			D1 10H	11,445	WT	WT	WT	23,875	ND
	1010	1A	D-7	1,323,535	WT	WT	WT	41,059	ND
	1011	1B	D-7	2,515,642	WT	WT	WT	494	ND
	1012	1A	D-7	1,530,810	WT	WT	WT	47,516	ND
			D1 10H	29,674	WT	WT	NA	72,019	21,895
	1013	1A	D-7	540,776	WT	WT	WT	174,345	77,401

	Patient	Genotype/S ubtype	Timepoint	Plasma		Liver			
				HCV RNA (IU/mL)	TVR Resistant Variants Observed	Primary Liver Sample		2nd Liver Sample	
						HCV RNA (copies/total RNA)	TVR Resistant Variants Observed	HCV RNA (copies/total RNA)	TVR Resistant Variants Observed
			D1 10H	112,185	WT	492,869	WT	73,089	WT
	1014	1A	D-7	2,937,704	WT	83,466	WT	77,882	WT
			D1 10H	45,201	WT	8,142	WT	106,888	WT
	1015	1B	D-7	2,518,054	WT	114,019	WT	ND	ND
			D1 10H	39,943	WT	106,458	WT	ND	ND
	1016	1B	D1 10H	23,407	WT	55,800	WT	ND	ND