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Early hepatitis C viral kinetics correlate with long-term outcome in patients receiving high dose induction followed by combination interferon and ribavirin therapy ☆

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See Editorial, pages 151–153

***Background/Aims*: The majority of patients with genotype 1 do not respond to interferon (IFN) plus ribavirin. Limited data exist on the use of induction followed by combination therapy.**

***Methods*: In this prospective study of 28 patients infected with genotype 1, randomization involved either daily or twice daily high dose IFN for 6 weeks, followed by standard therapy of 3 million units three times a week in combination with ribavirin for an additional 42 weeks. Hepatitis C virus (HCV) RNA was quantitated before and frequently during treatment.**

***Results*: The best correlate of response was δ (the infected cell loss rate). Sixteen patients continued on the study because they had at least a 2 log drop in their HCV RNA levels by week 12; all but one were PCR negative for HCV RNA at 48 weeks, and 14 of these 16 patients continued to be PCR negative at 72 weeks. Both African-Americans in our trial failed to respond to therapy, and differences were evident during the induction phase.**

***Conclusions*: This randomized study of induction IFN therapy followed by combination IFN plus ribavirin yielded the highest rate of sustained response (50%) reported to date in chronically HCV-infected patients with genotype 1. The predictive value of the infected cell loss rate needs to be evaluated prospectively in larger studies, particularly in patients receiving pegylated IFN.**

***Keywords*: Early hepatitis C viral kinetics; Long-term outcome; High dose induction; Combination interferon and ribavirin therapy**

1. Introduction

Approximately 2–3% of the world's population is infected with hepatitis C virus (HCV); HCV infection is one of the leading causes of liver failure and cancer and is the single most common indication for liver transplantation [1,2]. Despite the past decade's notable advances in the treatment of HCV infection [3], patients infected with genotype 1 still have a limited chance of attaining a sustained response to current therapy [4].

Several lines of evidence provide the rationale for induction therapy, i.e. use of high dose daily interferon (IFN) for

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several weeks to months [5], in these patients. HCV has a high rate of turnover and an in vivo half-life of only a few hours [6]. Interferon-α (IFN-α) has a short half-life, i.e. approximately 8 h [7], leading to wide fluctuations in plasma drug concentrations during treatment. In patients receiving standard treatment three times weekly, an intermittent increase in viral load has been observed on treatment-free days. Following single doses of IFN-α, serum HCV RNA in patients with genotype 1 can be reduced more effectively with higher doses of the drug [8]. Moreover, the antiviral effectiveness of IFN in blocking virus production and the free virion clearance rate have been shown to be significantly lower for genotype 1 than for non-genotype 1 patients [9], suggesting that more aggressive treatment is required in the case of genotype 1 infections. Furthermore, in theory, early clearance of HCV may prevent the development of treatment-resistant quasispecies [10].

Although most trials suggest that induction leads to higher end-of-treatment response rates, these improved rates have not translated into sustained response rates measured 6 months after cessation of therapy [5] when monotherapy has been used. Based on these considerations, we designed a randomized, controlled pilot trial in patients with genotype 1 infection to evaluate the effect on acute phase viral kinetics and long-term outcome of daily vs. twice daily induction IFN-α monotherapy for 6 weeks followed by combination therapy of IFN at standard doses with ribavirin for an additional 42 weeks.

2. Methods

2.1. Patients, scheme of treatment, and sampling

Informed consent was obtained from each patient included in the study, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the human research committees and institutional review boards of Oregon Health & Science University and Portland VA Medical Center. Adult patients who met the following inclusion criteria were considered for this study: not previously treated with IFN, a positive test for anti-HCV antibody, a positive serum HCV RNA level measured by a reverse transcription-polymerase chain reaction (RT-PCR) assay [11], HCV genotype 1 as assessed by the line probe assay [12], a serum alanine aminotransferase (ALT) concentration above the upper limit of normal, and findings on liver biopsy consistent with a diagnosis of chronic hepatitis C infection.

Twenty-eight patients were admitted to the General Clinical Research Center at Oregon Health & Science University, and a small venous catheter was placed for blood draw. To assign randomization, we used cards inscribed with 'QD' or 'BID' and placed inside opaque envelopes. Patients were randomly assigned to receive one of two possible IFN monotherapy (IFN-α2b, Schering-Plough Pharmaceuticals, New Jersey) regimens: 10 million units (MU) subcutaneous daily for 7 days, followed by 5 MU daily for an additional 5 weeks or 5 MU subcutaneous bid for 7 days, followed by 2.5 MU twice a day for an additional 5 weeks. Blood (1 ml) was drawn every hour for the first 60 h, and at days 8, 15 and 43, and then monthly with every clinic visit until 48 weeks and then at 72 weeks. Serum was separated within 4 h of venipuncture, aliquoted, and stored at −70 °C. After 6 weeks, all the patients received standard IFN (3 MU) thrice weekly in combination with ribavirin. Ribavirin was given orally in divided does of 1000 mg (weight <75 kg) or 1200 mg (weight >75 kg). At 12 weeks, if there had not been at least a 2 log drop in viral load from baseline, it was assumed that therapy had failed [13], and patients were discontinued from the study. Those patients that remained in the study received therapy for a total of 48 weeks (6 weeks of induction monotherapy and 42 weeks of standard combination therapy). Individuals were assayed again at 72 weeks, about 6 months after stopping therapy, to assess the sustainability of the response.

2.2. HCV RNA quantification

HCV RNA levels were quantified by the branched-DNA method (Quantiplex 2.0, Chiron, Emoryville, CA) and by an end point dilution PCR method [14]. Results from the branched DNA assay were used only if the viral load was above the sensitivity level of 2×10^5 eq/ml for all samples in that period. Results from the end point dilution PCR assay were used otherwise. In addition, HCV RNA levels were independently assessed by the National Genetics Institute (NGI, Culver City, CA) RT-PCR assay at baseline, and at weeks 6, 12, 48, and 72 (lower limit cut-off of 100 copies/ml) [11].

2.3. Mathematical model for estimation of HCV kinetics

To estimate the parameters characterizing viral decay under IFN-α therapy we used the model described by Neumann et al. [6]. In brief, free virus, V, infects uninfected hepatocytes generating infected cells, which in turn produce more virus. Virus is cleared with rate constant c and infected cells are lost at rate δ per cell. Initially, the patient is assumed to be in steady state with a constant baseline viral load, V_0. IFN-α is assumed to act in a dose-dependent manner by blocking a fraction, ε, of the production of virus from infected cells. We also assume that due to homeostasis the number of uninfected cells remains approximately constant during therapy. Under these assumptions, a set of differential equations can be constructed, which describe the model, and whose solution (see Neumann et al. [6]) predicts that the viral load, $V(t)$, will decrease with time on therapy, t, according to

$$V(t) = \frac{1}{2} V_0 \left[\left(1 - \frac{c + \delta - 2\varepsilon c}{\theta}\right) e^{-\lambda_1 (t-\tau)} + \left(1 + \frac{c + \delta - 2\varepsilon c}{\theta}\right) e^{-\lambda_2 (t-\tau)} \right]$$

where λ_1, λ_2 (the eigenvalues) are given by $1/2(c + \delta + \theta)$ and $1/2(c + \delta - \theta)$, respectively, and $\theta = \sqrt{(c - \delta)^2 + 4(1 - \varepsilon)c\delta}$. The model incorporates a delay, τ, between administration of therapy and its effect in blocking virion production. This solution is valid for $t > \tau$. For $t < \tau$, $V(t) = V_0$, where V_0 is the initial viral load.

In general, the decay profiles of virus under therapy show a biphasic decline. The slope of the first phase is characterized by c, while the slope of the second phase is characterized by δ. The half-life of free virus is $\ln(2)/c$, and the half-life of infected cells is $\ln(2)/\delta$, where $\ln(2) = 0.693$ is the natural logarithm of 2. Using data obtained during the 6 week induction period, when measurements were taken frequently, we estimated the parameters of the model by non-linear least-squares regression analysis. The delay, τ, was fixed at 8 h, the value determined by Neumann et al. [6], except for patient 24 where the data suggested a delay of 1 day (see Fig. 1).

3. Results

Twenty-eight patients with chronic HCV genotype 1 infection were enrolled in this prospective study. The baseline clinical, demographic, virological, and histological features of the daily (QD) group and twice daily (BID) group were comparable and are shown in Table 1. Twenty-three patients had pretreatment viral loads greater than 2×10^6 eq/ml, 11 assigned to the QD group and 12 assigned to the BID group. There was no correlation with patient weight and outcome, whether weight was considered a continuous or categorical variable.

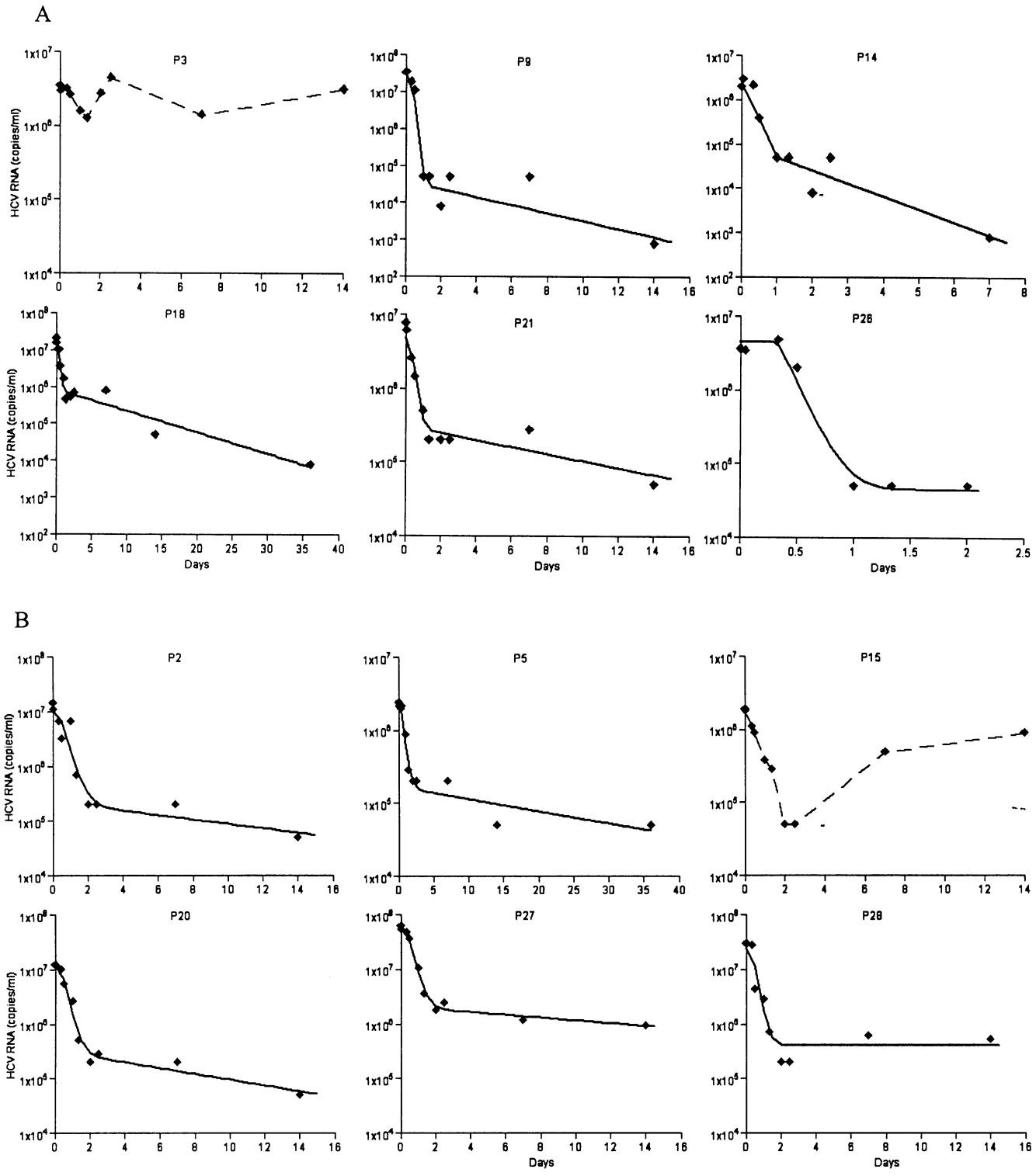

Fig. 1. Kinetics of HCV RNA change in individual patients. (A) Six representative individuals of the QD group. (B) Six representative individuals of the BID group. The symbols are the experimental data, and the solid lines indicate the best fit of the kinetic model. Dashed lines were used in those cases where a kinetic analysis was not done (P3 and P15 correspond to the African-Americans in our study).

3.1. Induction phase

The HCV RNA level in serum was quantified according to the procedures described in Section 2. The results were fitted using non-linear regression techniques as previously described [6]. Three of the 28 patients had poor virological response profiles (P3, P15, and P16), in which the HCV RNA level declined for 2 days or less and then returned to

Table 1
Pretreatment features of patients assigned to QD vs. BID induction dosing

	Daily induction	Twice daily induction
Number of patients	14	14
Age (years, mean ± SEM)	43.8 ± 1.8	42.3 ± 1.5
Male/female	9/5	12/2
Body weight (kg)	79.7	86.4
HCV RNA[a] (eq/ml) (mean ± SEM)	$15.0 \pm 4.5 \times 10^6$	$17.8 \pm 4.7 \times 10^6$
HCV genotype		
1a	10	12
1b	4	1
Mixed		1
ALT[b] (IU/l) (mean ± SEM)	85.0 ± 11.4	136.3 ± 28.7
AST[b] (IU/l) (mean ± SEM)	50 ± 7.0	72.9 ± 13.0
Fibrosis (≥3 subscore)[c]	3 (21.4%)	4 (28.6%)
Non-Caucasian	1	1

[a] bDNA, branched DNA quantitative assay.
[b] ALT, alanine aminotransferase; AST, aspartate aminotransferase.
[c] Bridging fibrosis or cirrhosis.

baseline (Fig. 1). These patients were not included in the dynamic analysis. It is interesting to note that the two African-Americans (P3 and P15) were among the three in this group. Also, for P17, who required IFN dose reduction for leukopenia (absolute neutrophil count $\leq 500/mm^3$), we only used the data before therapy was changed.

After initiation of induction therapy, the viral load remained constant for about 8 h, followed by a biphasic response, as previously described [6]. The first phase decline was very rapid in nearly all patients, with estimated free virion half-lives ($t_{1/2}$) between 1 and 7.5 h (Table 2). There was one outlier, P8 in the QD group, $t_{1/2} = 0.2$ h. The first phase decline was faster in the once daily group than in the BID group ($t_{1/2} = 2.0$ vs. 3.9 h) but not significantly so when the outlier was excluded ($P = 0.064$, Wilcoxon rank sum test).

Sixteen patients showed a second phase decline, with estimated infected cell half-lives between 1 and 16.9 days. It is interesting to note that, within this group of patients, the decay was not significantly different between the QD and the BID groups ($t_{1/2} = 5.9$ vs. 8.8 days, $P = 0.169$, Wilcoxon rank sum test). For eight patients we could not quantify a second phase decline (five in the QD group and three in the BID group). In addition, one individual (P17) needed dose reduction for leukopenia at day 4, thus precluding the possibility of quantifying the second phase kinetics.

Of the 26 patients who completed the 6 week induction period, only seven (27%) were PCR negative for HCV RNA at that time by the NGI assay. Further, there was no correlation between the PCR status at week 6 and the treatment dose protocol (logistic regression, $P = 0.18$). In fact, there was no difference in the percentage reduction in viral load between the two dosing groups (99.1 vs. 98.7% in the QD and BID groups, respectively, $P = 0.23$, Wilcoxon rank sum test, without the African-Americans).

3.2. Long-term response

After the 6 week induction period, all patients received standard therapy of 3 MU IFN three times a week in combination with ribavirin. Seven patients experienced a viral rebound between weeks 6 and 12 of the study. This rebound was not associated with the treatment protocol of the individual or any of the kinetic parameters (logistic regressions, $P > 0.10$).

By 12 weeks, ten individuals were discontinued from the study, because they did not show a 2 log or greater drop in viral load from baseline (Table 2). Of the individuals that experienced a viral load rebound in this period, only one remained in the study because his viral load was still below 2 log of baseline (P22). The 16 patients that continued on the study were given standard therapy for an additional 36 weeks, and all but one (P13) were PCR negative for HCV RNA at 48 weeks. Most importantly, this high level of response to treatment was sustainable, since at 72 weeks, 6 months after stopping therapy, 14 of these 16 patients continued to be PCR negative (P13 and P21 were positive). These levels of response were not associated with the induction dose schedule of the patients ($P = 0.27$ at 48 weeks, and $P = 0.11$ at 72 weeks). Significantly, the protocol used here, with an induction phase, reached a 50% sustained response rate (14 of the 28 individuals enrolled in the study), and there was no preference of one dosing protocol over the other.

The virological response (PCR negative or positive for viral RNA) at week 6 showed a significant association with the loss rate of infected cells, δ, and at week 12 it approached, but did not reach, significance. The individuals with faster second phase declines, which presumably reflects increased clearance of virally infected cells, had a higher probability of being PCR negative (logistic regression $P = 0.005$ and $P = 0.085$ at 6 and 12 weeks, respectively). In the long-term analysis, at 48 (72) weeks, 15 (14) out of 16 individuals still in the study were HCV RNA PCR negative and hence there were insufficient data for a comparison between long-term response outcome and second phase slope.

We did not observe any significant relationship of a positive or negative viral response with the first phase parameter c or the drug efficacy ε. In addition, we found no correlation between any of the kinetic parameters and the baseline levels of ALT or AST. Thus, the most significant predictor of response was δ measured at 6 weeks.

3.3. Safety, withdrawal from the study

Side effects and tolerance were similar in both treatment groups. Only two patients in the study (one in each treatment group) dropped out before completion of the induction phase and one additional patient dropped out at 20 weeks

Table 2
Kinetic analysis and long-term outcome of therapy for individuals in the QD (top) and BID (bottom) protocols[a]

Patient	V_0 ($\times 10^6$ copies/ml)	First phase $t_{1/2}$ (h)[b]	Second phase $t_{1/2}$ (days)	Efficacy ε (%)	RNA status (weeks)[c]			
					6	12	48	72
QD								
P1	49.4	1.5	ND	99.9	+	−	−	−
P3	3.5	ND	ND	ND	+	+ /D		
P8	0.5	0.2	ND	90.2	+	+ /D		
P9	32.9	1.6	2.8	99.9	−	−	−	−
P10	6.3	3.4	16.1	90.8	D			
P12	2.3	2.2	ND	97.2	+	+ /D		
P13	9.1	1.2	4.1	99.4	−	−	+	+
P14	2.3	1.4	1.0	97.0	−	−	−	−
P16	2.4	ND	ND	ND	−	−	−	−
P18	13.8	2.9	4.9	94.5	+	−	−	−
P19	61.5	1.5	ND	99.9	+	−	−	−
P21	4.7	2.9	6.0	94.0	+	−	−	+
P25	22.8	3.0	6.2	98.5	−	−	−	−
P26	4.4	2.2	ND	99.0	+	+ /D		
Mean	15.4	2.0	5.9	96.7				
SD	19.4	0.9	4.9	3.5				
BID								
P2	10.1	6.4	7.3	98.0	+	−	−	−
P4	10.6	1.9	3.1	90.9	−	−	−	−
P5	2.4	7.5	16.9	93.4	+	+	−	−
P6	4.0	1.4	ND	98.8	+	+ /D		
P7	28.7	1.0	ND	99.5	−	−	−	−
P11	28.2	1.7	14.3	99.8	+	−	−	−
P15	1.9	ND	ND	ND	+	+ /D		
P17	10.3	6.6	ND	92.8	+	+ /D		
P20	11.6	5.0	5.5	97.5	+	+	−	−
P22	24.6	2.2	8.7	99.6	+	+	−	−
P23	10.2	3.4	9.1	92.0	+	+ /D		
P24	1.9	4.2	3.1	75.9	+	+ /D		
P27	56.7	5.3	11.3	96.5	+	+ /D		
P28	23.6	3.9	ND	98.3	D			
Mean	16.1	3.9	8.8	94.8				
SD	15.1	2.2	4.8	6.5				
P value	0.568	0.036	0.169	0.463				

[a] Results of fitting the HCV RNA decline kinetics to the model of Neumann et al. [6] are summarized by giving the half-lives for the first phase decline, corresponding to virion removal, the half-lives for the second phase decline, corresponding to infected cell loss, and the efficacy of IFN in blocking virion production (ε). The long-term outcome of therapy is indicated by the HCV RNA PCR status positive (+) or negative (−) at the time points indicated. *P* values are for differences between the two groups, determined with a Wilcoxon rank sum test. ND, not determined; D, discontinued.

[b] First phase $t_{1/2} = 0.693/c$, second phase $t_{1/2} = 0.693/\delta$.

[c] HCV RNA status (by NGI RT-PCR assay) at these time points.

because of anxiety. Hypothyroidism developed in two patients and required hormone replacement without need for IFN dose reduction.

4. Discussion

In the current study, we investigated the dynamics of HCV viral clearance and sustainability of long-term responses of daily vs. twice daily IFN-α administered for a 6 week induction period, followed by 42 weeks of standard IFN plus ribavirin therapy. To our knowledge, this study is the first to specifically examine the effect of early viral kinetic parameters on sustained long-term response to combination therapy. Our analyses of the induction period (based on hourly blood draws for the first 60 h) confirmed the biphasic viral decline previously described for a majority of patients. In our study, the virion clearance rate, which influences the rapid first phase kinetics, was higher in the QD group. However, if one outlier is excluded the difference in estimated clearance rate constants between the QD and BID groups is not significant. The infected cell loss rate, δ, influences the long-term decline of virus. Of those patients with a measurable second phase decline, there

was no significant difference in δ between the groups. Accordingly, a recent short-term analysis of daily vs. twice daily IFN-α for 4 weeks (in combination with ribavirin) [15] in 24 patients failed to support the hypothesis that twice daily dosing is more effective.

From pharmacokinetics one might expect BID dosing to be more effective than QD dosing since the concentration of IFN should remain more uniform. However, there are factors that complicate this interpretation. The effect of IFN-α is mediated by the binding of IFN to type I IFN receptors [16]. The amount of receptor binding and signaling thus depends on the concentration of IFN. Hence, one should expect the QD group, which is given twice the dosage of IFN in the initial injection (e.g. 10 MU once a day vs. 5 MU twice a day), to have more IFN receptors bound. This could translate into a larger antiviral effect, as suggested by the work of Neumann et al. [6]. In addition, the total daily amount of IFN given is the same in both dosing groups. Thus, at least for the first 12 h each day the QD group is expected to be better suppressed than the BID group. During the second 12 h the BID group might have an advantage if receptor engagement needs to persist, but interestingly for the full 24 h period our results suggest that the total effect of the two dosing schedules is about the same.

Of interest, a recent study by Shiratori et al. [17] demonstrated improved antiviral efficacy with twice-a-day vs. daily intravenous IFN-β. However, several aspects of that study need to be addressed. They used smaller doses, a total daily dose of 6 MU vs. 10 MU in the current study, and the doses were delivered intravenously rather than subcutaneously. Given the relatively short half-life of IFN in the organism, it is possible that intravenous IFN-β at the 6 MU dose will fall below the minimum inhibitory concentration sometime before the next dose. On the other hand, the higher dosage of IFN-α (10 MU) might be able to sustain the serum level of IFN for a longer period. In this regard, it is worth noting that in the Shiratori et al. study, the difference detected was at the level of drug efficacy (ε), with the twice daily protocol being more efficacious, whereas here we do not find any difference in this parameter between the two regimens. Although the more frequent dosing of IFN-β was associated with a more efficient virological response from patients, it also resulted in considerably higher rates of toxicity. BID vs. QD dosing was equally well tolerated in the current study; moreover, there was no difference in the long-term outcome between our two therapy protocols. Of note, the overall rate of side effects and drop-out rate was comparable to prior studies with standard combination therapy [4].

In this study we conducted a short- and long-term assessment of the sustainability of the response to treatment. At 12 weeks, 16 of the 26 patients who completed the induction phase were HCV RNA negative by PCR. These patients continued therapy to 48 weeks, at which point 15 of the 16 were negative. The large proportion of patients reaching early viral negativity theoretically allows for a high rate of end-of-treatment response [15]. We designed the study based on the 12 week 'stopping rule' which had been adopted widely in clinical practice based on monotherapy data [13]. In retrospect, however, it is possible that a proportion of the ten patients who were dropped at 12 weeks because of non-response would have eventually become sustained responders. Accordingly, a recent large analysis of combination therapy showed that discontinuing treatment based on a positive week 12 HCV RNA determination would potentially deny effective continuation of therapy to 11% of patients [18].

The rates of virologic breakthrough and relapse were very low in our current study. Only one patient (P13, Table 2) who was PCR negative at 12 weeks demonstrated biochemical and virologic breakthrough while on therapy at week 40. Moreover, 6 months after cessation of therapy (at 72 weeks), 14 of the 16 patients were still negative for HCV RNA. This is a 50% rate of response in relation to the 28 subjects who started the study and an 87.5% rate in relation to the 16 patients who responded well to the induction period. These rates are much higher than previously described for genotype 1 with standard combination therapy [4,19]. In our study, the 6.7% (1/15) relapse rate off therapy following an end-of-treatment response was much lower than the 24.3 and 18.6% rates reported in patients treated with standard IFN–ribavirin [4,18,20]. Similarly, in a preliminary report by Larsen et al. [21], induction therapy with either 5 or 10 MU followed by combination therapy was associated with significantly improved response rates in genotype 1 patients, and relapse was not observed. The reasons for the very low relapse rates are unclear, and we can only speculate about the origin of this effect. During induction, the high doses of IFN used might better penetrate privileged sites of HCV replication and lead to better antiviral control. Moreover, the high viral loads seen in HCV infection may lead to viral suppression of host immune responses. Rapidly reducing the viral levels by induction dosing might then remove this suppression more effectively than maintenance dosing, or alternatively, since IFN is an immune stimulator, high induction doses might provide a better host-dependent immunomodulatory effect. Moreover, it is of considerable interest that both African-Americans in our trial failed to respond to therapy (P3 and P15), and when compared to the other patients, differences in viral kinetics were evident during the induction phase. These findings confirm and extend recent reports [22] demonstrating relative resistance to antiviral therapy in African-Americans, and a National Institutes of Health-sponsored, multicenter study is underway to address the potential mechanisms underlying the differences between African-Americans and non-African-Americans.

In summary, the failure of the standard thrice weekly combination regimen to achieve sustained responses in HCV genotype 1-infected Caucasian patients can be overcome with a 6 week induction phase of IFN. The predictive

value of the infected cell loss rate must be confirmed prospectively in a larger number of patients and in particular, with the newer longer-acting forms of IFN [23,24].

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