Pharmacodynamics of PEG-IFN α Differentiate HIV/HCV Coinfected Sustained Virological Responders From Nonresponders

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Pegylated interferon (PEG-IFN) has become standard therapy for hepatitis C virus (HCV) infection. We evaluated whether PEG-IFN pharmacodynamics and pharmacokinetics account for differences in treatment outcome and whether these parameters might be predictors of therapeutic outcome. Twenty-four IFN-naïve, HCV/human immunodeficiency virus–coinfected patients received PEG-IFN α-2b (1.5 μg/kg) once weekly plus daily ribavirin (1,000 or 1,200 mg) for up to 48 weeks. HCV RNA and PEG-IFN α concentrations were obtained from samples collected frequently after the first 3 PEG-IFN doses. We modeled HCV kinetics incorporating pharmacokinetic and pharmacodynamic parameters. Although PEG-IFN concentrations and pharmacokinetic parameters were similar in sustained virological responders (SVRs) and nonresponders (NRs), the PEG-IFN α-2b concentration that decreases HCV production by 50% (EC_{50}) was lower in SVRs compared with NRs (0.04 vs. 0.45 μg/L [$P = .014$]). Additionally, the median therapeutic quotient (*i.e.*, the ratio between average PEG-IFN concentration and EC_{50} [$\bar{C}/EC_{50}$]), and the PEG-IFN concentration at day 7 divided by EC_{50} ($C(7)/EC_{50}$) were significantly increased in SVRs compared with NRs after the first (10.1 vs. 1.0 [$P = .012$], 2.8 vs. 0.3 [$P = .007$], respectively) and second (14.0 vs. 1.1 [$P = .016$], 5.4 vs. 0.4 [$P = .02$], respectively) PEG-IFN doses. All 3 parameters may be used to identify NRs. In conclusion, PEG-IFN concentrations and pharmacokinetic parameters do not differ between SVRs and NRs. In contrast, pharmacodynamic measurements—namely EC_{50}, the therapeutic quotient, and $C(7)/EC_{50}$—are different in coinfected SVRs and NRs. These parameters might be useful predictors of treatment outcome during the first month of therapy. (HEPATOLOGY **2006;43:943-953.**)

Hepatitis C virus (HCV) and human immunodeficiency virus (HIV) are both parenterally transmitted viruses that coinfect more than 250,000 individuals in the United States.[1] In HCV monoinfected patients, viral eradication occurs in approximately 55% of those treated with current standard therapy: weekly pegylated interferon (PEG-IFN) in combination with daily ribavirin (RBV).[2,3] In HIV/HCV–coinfected patients, successful therapeutic outcomes are substantially lower, ranging from 27% to 40% overall and from 14% to 29% for difficult-to-treat genotype 1 patients.[4-6] Consequently, understanding why HIV/HCV–coinfected patients respond to current treatment and how to improve responses is of clinical importance. A fundamental question is whether increased serum IFN α concentrations result in improved therapeutic outcomes.

One method of assessing the effectiveness of anti-HCV treatment is the analysis of HCV RNA decay using mathematical models.[7-11] These models have generally not incorporated the effects of time-varying IFN concentrations. PEG-IFN α-2b concentration, however, wanes to a

Abbreviations: PEG-IFN, pegylated interferon; HCV, hepatitis C virus; SVR, sustained virological responder; NR, nonresponder; HIV, human immunodeficiency virus; RBV, ribavirin; ETR, end-of-treatment responder.

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considerably greater degree between doses than does standard, daily IFN.[9,12-14] To account for waning PEG-IFN α concentration between doses, HCV kinetic models that incorporate pharmacodynamic and pharmacokinetic parameters should be used to analyze data from PEG-IFN–treated patients.[9]

In this study, we evaluated whether serum PEG-IFN α-2b pharmacodynamic and pharmacokinetic parameters are associated with improved treatment responses in a cohort of HIV/HCV–coinfected patients and whether these parameters might be predictors of treatment outcome. We measured HCV RNA and serum PEG-IFN α concentrations frequently after each of the initial 3 doses of PEG-IFN α in 24 coinfected patients treated with PEG-IFN α-2b (12 kd) and ribavirin (RBV). We analyzed these data using HCV dynamic models that incorporate pharmacodynamic and pharmacokinetic parameters. Applying the data from coinfected patients to these models, we found that EC_{50} (*i.e.*, the serum PEG-IFN concentration that results in a 50% reduction in HCV RNA during the first phase) was significantly lower in sustained virological responders (SVRs) compared with nonresponders (NRs). Additionally, the therapeutic quotient (*i.e.*, the ratio between the average PEG-IFN concentration and EC_{50} [$\bar{C}/EC_{50}$]), as well as the PEG-IFN concentration at day 7 divided by EC_{50} ($C(7)/EC_{50}$) was significantly increased in SVRs compared with NRs. All 3 parameters might be useful predictors of treatment outcome during the first month of therapy.

Patients and Methods

Study Participants

Twenty-four HCV/HIV–coinfected, PEG-IFN α/RBV treatment–naïve patients from the Hepatitis Clinic of New York/Weill/Cornell Medical Center were enrolled in a prospective trial to evaluate the pharmacokinetics and pharmacodynamics of PEG-IFN α-2b. At the time that this study was initiated, PEG-IFN α-2b was the only PEG-IFN formulation approved by the US Food and Drug Administration. The Institutional Review Board of the Weill Medical College of Cornell University approved the study, and written informed consent was obtained from all patients. Inclusion criteria required that patients had detectable HCV RNA, were on a stable antiretroviral regimen of US Food and Drug Administration–approved agents or no antiretroviral agents for at least 4 weeks prior to PEG-IFN/RBV initiation, and had a $CD4^+$ T cell count of 100 cells/mm^3 or more. Patients were excluded if they had prior treatment with IFN α or RBV, any cause of liver disease other than chronic HCV infection, severe depression, active substance abuse, or immunodeficiency-related opportunistic infections or were pregnant or lactating. Study entry required a liver biopsy, which was graded and staged using the Scheuer system.[15] Subjects were treated with standard care at the initiation of the study: PEG-IFN α-2b 1.5 μg/kg once weekly and RBV 1,000-1,200 mg daily (Schering Plough Corporation, Kenilworth, NJ) for up to 48 weeks (Table 1). Patients with HCV RNA below detection at the end of treatment were end-of-treatment responders (ETRs), and those with absent HCV RNA 24 weeks posttreatment were SVRs. To obtain a balanced racial mix, we enrolled 12 African American patients and 12 Caucasian patients (Table 1).

Sample Acquisition

Patients were admitted to the General Clinical Research Center at New York/Weill/Cornell Medical Center for the first and second doses of PEG-IFN α-2b, which were administered by a health care professional. Participants had HCV RNA determinations at baseline, after the first dose (6, 12, 24 h) and on days 2, 3, 5, 6, and 7; after the second dose (6, 12, 24 h) and on days 9 and 14; and after the third dose on days 15 and 16. Subsequently, patients returned monthly for the first 3 months and every 6 weeks thereafter. After medication discontinuation, patients returned at 4, 12, and 24 weeks.

HCV and HIV RNA Measurements

For HCV RNA measurements, total RNA was initially extracted from the sample. Subsequently, virus was quantitated using a validated real-time reverse-transcriptase polymerase chain reaction assay with a lower limit of detection of 29 IU/mL (Schering Plough Research Institute). The amplification target was the 5′-UTR of the HCV genome. An internal RNA control was added to each sample to assess the efficiency of RNA extraction and the reverse-transcriptase polymerase chain reaction. Appropriate positive and negative controls were added to each assay run. HIV RNA was measured using the Roche COBAS Amplicor HIV-1 MONITOR assay version 1.5 (Roche Diagnostics, Branchberg, NJ).

HCV Genotyping

HCV genotypes were determined via sequencing of the NS5B region[16] and were classified according to the method of Simmonds and colleagues.[17,18]

PEG-IFN α Electrochemiluminescence Assay

Serum samples were assayed for PEG-IFN α-2b using a validated, electrochemiluminescence-based assay and an ORIGEN analyzer (IGEN International, Inc., Gaithersburg, MD).[12] The assay had a lower limit of detection of 0.05 μg/L.[12]

Table 1. Baseline Characteristics

Patient No.	Race	HCV RNA (log_{10} IU/mL)	Inflammation	Fibrosis	ALT (IU/L)	Genotype	HIV RNA (copies/mL)	CD4 (cells/μL)	Weight (kg)
3	AA	6.68	0	0	119	1b	<400	460	88.0
4	AA	6.39	2.5	2	52	1a	<400	472	81.6
6	AA	7.02	2.5	2	197	1b	1,641	258	106.6
12	AA	5.73	2	1	108	1b	3,391	449	70.5
16	AA	4.71	2	1-2	299	1a	<400	493	83.0
17	AA	6.74	2.5	2-3	119	1a	28,807	772	79.8
19	AA	6.24	3	3-4	119	1a	31,106	223	77.1
26	AA	5.75	2	2	60	2b	8,772	810	74.8
32	AA	6.75	2	2	64	1a	<400	646	89.8
36	AA	5.09	2	2	33	1a	<400	506	84.8
53	AA	6.14	1	2	23	1a	<400	498	85.3
503	AA	5.16	3	3-4	233	1a	<400	319	65.8
1	C	6.07	2	3	29	1a	<400	187	81.6
2	C	6.52	1-2	3	42	1a	<400	434	88.9
5	C	6.68	4	3	111	1b	<400	319	95.3
7	C	5.50	3	2	316	1a	541	521	82.6
9	C	6.93	2	2-3	113	1a	<400	1,193	94.8
10	C	5.44	2	2-3	34	1a	<400	209	81.6
31	C	6.34	2	3	25	1a	60,774	462	83.5
34	C	6.71	2-3	3	105	1a	<400	495	83.0
64	C	6.85	2	2-3	66	1b	200,036	483	69.9
66	C	5.32	2-3	2-3	48	1a	<400	490	84.4
502	C	4.58	4	4	173	3a	<400	404	62.6
505*	C	7.09	1	1	38	2	<400	334	93.3

NOTE. Inflammation and fibrosis were assessed using the Scheuer system.
Abbreviations: HCV, hepatitis C virus; ALT, alanine aminotransferase; HIV, human immunodeficiency virus; AA, African American; C, Caucasian.
* Received 32 weeks of PEG-IFN/RBV therapy.

Mathematical Modeling

Viral Dynamics. As detailed previously,[9,19] we extended the Neumann et al. model of viral dynamics[7] by allowing the drug effectiveness, ε, to vary with PEG-IFN concentration.

Pharmacokinetics. We assume the PEG-IFN dose, D, reaches the injection site immediately, is absorbed into the blood with rate constant k_a, is eliminated from the blood with rate constant k_e, and is distributed through a volume V_d. Only a fraction, F, of the drug is bioavailable. Using a standard 1-compartment pharmacokinetic model (see Powers et al.,[9] Gabrielsson,[20] and Welling[21] for details), the serum PEG-IFN concentration, $C(t)$, after the first dose given at time $t = 0$, and before the second dose, is

$$C(t) = \frac{FD}{V_d}\frac{k_a}{k_e - k_a}[e^{-k_a t} - e^{-k_e t}].$$

According to this equation, $C(t)$ rises to a maximum concentration, C_{max}, before decreasing again until the next dose of PEG-IFN. A new dose is given on day 7, and we find[9,19]

$$C(t) = C(7)e^{-k_e(t-7)} + C_i(7)\frac{k_a}{k_e - k_a}(e^{-k_a(t-7)} - e^{-k_e(t-7)})$$

where $C(7)$ is the drug concentration in serum at day 7, $C_i(7) = FD(1 + e^{-7k_a})/V_d$, and t varies between days 7 and 14.

Effectiveness and the Combined Viral Dynamic/Pharmacodynamic Model. The changes in PEG-IFN serum concentration, $C(t)$, lead to changes in effectiveness. To account for this variation in PEG-IFN effectiveness, we used the standard pharmacodynamic model[19-21]

$$\varepsilon(t) = \frac{C(t-\tau)^n}{EC_{50}^n + C(t-\tau)^n},$$

for $t > \tau$ and $\varepsilon(t) = 0$ for $t < \tau$. Here EC_{50} is the concentration at which the drug's effectiveness $\varepsilon(t)$ is half its maximum, and n is the Hill coefficient, a parameter that determines how steeply the effectiveness rises with increasing concentration (Fig. 1). The parameter τ represents a delay between the time when PEG-IFN binds its receptor and when it begins its biological action; that is, effectiveness at time t depends on concentration at $t - \tau$.

To create a combined viral dynamic/pharmacodynamic model, we substituted the effectiveness function $\varepsilon(t)$ given by the equation above into the system of differential equations of the Neumann et al. model.[7] This approach gave better fits than those obtained using the original Neumann et al. model (which assumes constant ε), particularly in patients in whom HCV RNA re-

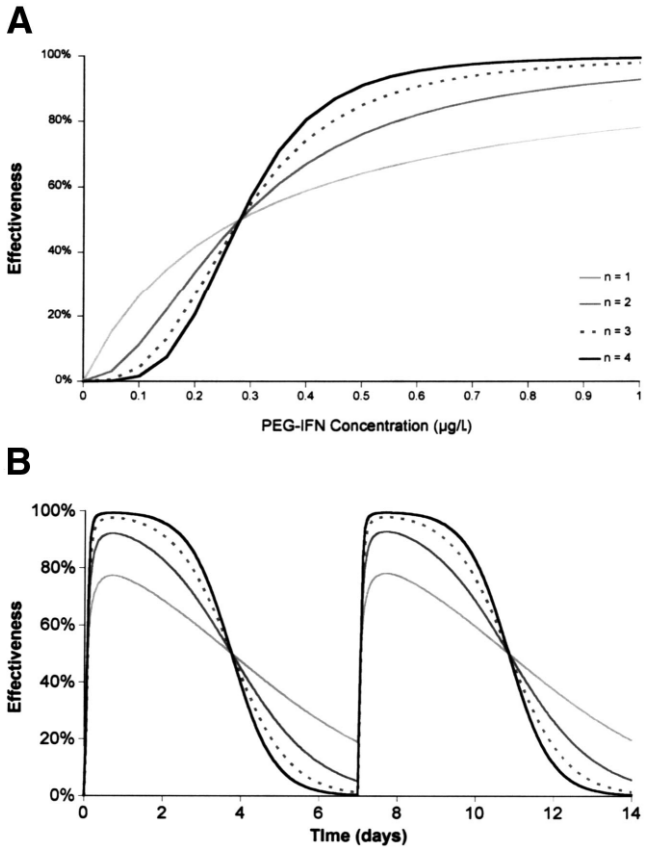

Fig. 1. Relationship between drug effectiveness and serum drug concentration. (A) For larger n (cf. $n = 4$) the effectiveness is either close to zero or near maximum depending on whether the drug concentration is above or below the EC_{50} (here $EC_{50} = 0.3$ μg/L). (B) The drug concentration varies between doses, first increasing and then decreasing. Thus, the effectiveness will change over time, and how sharply it varies depends on n. PEG-IFN, pegylated interferon.

bounded during the interval between doses (data not shown). The original Neumann et al. model predicts a monotonic decay of serum HCV RNA, and thus cannot fit the HCV rebound observed in many patients treated with PEG-IFN.

Data Fitting. Using nonlinear least-squares regression analysis based on a Levenberg-Marquardt algorithm, we fitted the pharmacokinetic model to the PEG-IFN concentration for the first and second weeks separately to gain the best possible representation for the PEG-IFN concentration $C(t)$. Thus, we estimated first- and second-dose values for k_a and k_e for each patient. Fitting the data with the same parameters for both doses resulted in poorer fits (data not shown). We treated FD/V_d as a single free parameter, except in cases where the resulting estimates of k_a and k_e were equal to one another. In these cases, FD/V_d was fixed at 1.515 μg/L, based on published values of F and V_d and the dose appropriate for the average patient weight.[12] When PEG-IFN concentration was below the detection limit of 0.05 μg/L, a value of 0.05 μg/L was used.

As described by Neumann et al.,[7] we first estimated the initial viral load, V_0, and initial delay before viral decay, t_0, by fitting a reduced model, with constant effectiveness, for the early phase of decay. These values, as well as the values of k_e, k_a, and FD/V_d estimated by the pharmacokinetic fits, were used in fitting the patients' HCV RNA data to the combined viral dynamic/pharmacodynamic model over the first 2 doses. With these fits, we estimated c, δ, and EC_{50}. The delay τ was assumed to be equal to the initial delay t_0. We fit each patient's data to models with the Hill coefficient, n, fixed at 1, 2, 3, or 4 to determine the value of n that produced the best fit (*i.e.*, the fit with the smallest residual sum of squares) for each patient. Data from patients who did not have an initial decline in HCV RNA levels (patients 3, 12, 31, and 36) could not be fitted to the combined viral dynamic/pharmacodynamic model. In our fitting procedure, parameter estimates were constrained to be nonnegative, and convergence was defined when the sum of squared residuals was not reduced by at least 10^{-9} from one iteration to the next. Also, for the parameters estimated by our fits, we calculated 95% CIs by nonparametrically resampling the residuals 500 times in each case. In some cases, particularly for k_a, the upper bound of the CI could not be reliably estimated. Thus, when this bound is larger than 100 days^{-1}, we indicate that it was not determinable.

Using the best-fit parameters, we calculated the theoretical PEG-IFN concentration, $C(t)$, HCV viral load, $V(t)$, and effectiveness, $\varepsilon(t)$. From these, we estimated the maximum drug concentration, C_{max}, the maximum effectiveness, ε_{max}, the average drug concentration, $\bar{C}$; (area under the PEG concentration curve, divided by 7 days), $C(7)/EC_{50}$, and the average effectiveness, $\bar{\varepsilon}$, for weeks 1 and 2.

Statistical Analysis

We used nonparametric methods to compare baseline, pharmacokinetic, and pharmacodynamic parameters of SVR with those of NR. To compare continuous variables, we used a 2-tailed Wilcoxon rank-sum test adjusted for the presence of ties. To compare categorical variables, we used a 2-tailed Fisher exact test (version 8.2; STATA Corporation, College Station, TX). In all cases, a P value of less than .05 was considered significant. Medians and interquartile ranges, the difference between the third and first quartile, were calculated for all pharmacokinetic and pharmacodynamic parameters. To evaluate pharmacodynamic parameters (EC_{50}, therapeutic quotient, and $C7/EC_{50}$) as predictors of treatment outcome, we calculated the positive and negative predictive values. We selected

cutoff values for each of these parameters to obtain a negative predictive value of 100% so that we would not misclassify potential SVR patients.

Results

Virological Response. Twenty-one patients completed the viral and pharmacokinetics portion of the study and provided frequent blood samples after each of the initial 3 doses of PEG-IFN. Three patients (5, 17, and 32) withdrew within the first 15 days because of treatment-related adverse effects or noncompliance. These 3 patients were excluded from the analysis of pharmacokinetic and pharmacodynamic parameters.

Six months after cessation of treatment, 6 patients (25%) were SVRs (2 African American, 4 Caucasian). Eight patients (33%) were ETRs (3 African American, 5 Caucasian). In all subsequent analyses, the 2 ETR patients who were not SVRs were included in the NR group. Among baseline characteristics, HCV RNA was significantly decreased in SVRs ($P = .007$) compared with NR and body weight tended to be increased ($P = .06$) in NR compared with SVR patients. $CD4^+$ cell counts, alanine aminotransferase levels, necroinflammation, fibrosis, and HIV RNA levels were not significantly different between the SVR and NR groups.

Drug Concentration and Viral Profiles. During the first 2 weeks of treatment, PEG-IFN α-2b concentration peaked and waned (Fig. 2). On average, serum PEG-IFN α-2b peaked 1.2 ± 0.58 days after the first dose at 0.90 ± 0.43 μg/L, and decreased to 0.13 ± 0.09 μg/L immediately preceding the second dose. A similar pattern was repeated in the second week; drug levels peaked 1.1 ± 0.81 days after the second dose at 0.94 ± 0.42 μg/L and then decreased to 0.20 ± 0.15 μg/L. The PEG-IFN profiles were highly variable between patients, although intrapatient variability was small when comparing weeks 1 and 2 (Fig. 2). The median PEG-IFN concentration during weeks 1 and 2 (for SVR 0.42 μg/L and 0.55 μg/L; and for NR 0.42 μg/L and 0.44 μg/L, respectively) (Table 2), as well as the time to maximum concentration at week 1 and 2 (mean 1.2 d), were similar in the SVR and NR patient groups, suggesting that PEG-IFN concentration per se does not correlate with treatment outcome.

Serum HCV RNA concentration changed inversely with PEG-IFN α-2b concentration during the initial 2 weeks of treatment. After a delay of 0.3 ± 0.2 days, HCV RNA declined to a minimum that was 1.3 ± 0.88 $\log_{10}$ below baseline at 2.8 ± 2.1 days. Subsequently, HCV RNA partially rebounded. Immediately preceding the second PEG-IFN dose, the mean HCV RNA was 0.72 ± 1.0 $\log_{10}$ below baseline. Both in SVR and NR, a new, fast decline ensued with subsequent doses of PEG-IFN, as each new dose re-established the high effectiveness of the drug.

Pharmacokinetic Parameters. We estimated PEG-IFN absorption and elimination rate constants for weeks 1 and 2. The absorption rate varied substantially among patients, while the elimination rate was relatively constant (Table 3). Aggregating data for weeks 1 and 2, the average rates were $k_a = 3.5$ days^{-1} and $k_e = 0.44$ days^{-1}, which are comparable to published values for PEG-IFN α-2b ($k_a = 3.6$ days^{-1} and $k_e = 0.42$ days^{-1}).[22] Neither k_a nor k_e were significantly different between the SVR and NR groups (Table 3). We also estimated FD/V_d, where D is the administered dose of PEG-IFN, F is its bioavailability, and V_d is the volume of distribution (see Patients and Methods). Again, the week 1 and 2 estimates for FD/V_d did not differ between the SVR and NR groups (Table 3).

Viral Kinetic and Pharmacodynamic Parameters. With each patient's pharmacokinetic parameters held constant, we estimated the model parameters c, δ, and EC_{50} by fitting both the initial decline and the viral rebound at the end of the week (Fig. 2). The median virion clearance rate did not differ significantly between the SVR and NR groups (Table 3). By contrast, the median infected cell loss rate, δ, is significantly faster in SVRs (0.51 vs. 0.12 days^{-1}; $P = .044$) compared with NRs.

To evaluate the potential differences in PEG-IFN α-2b effectiveness in SVRs and NRs, we estimated the EC_{50}, which is the PEG-IFN α-2b concentration necessary for half-maximum effectiveness (*i.e.*, for approximately a 50% decrease in HCV RNA during the first phase decline). The median EC_{50} is approximately tenfold smaller in SVRs than in NRs (0.04 vs. 0.45 μg/L; $P = .014$) (Table 3; Fig. 3A). An EC_{50} value of 0.5 has a positive predictive value of 46.2%, a negative predictive value of 100%, a sensitivity of 100%, and a specificity of 36.4%. Therefore, the proportion of patients with EC_{50} values of less than or equal to 0.5 who are SVRs is 46.2%, and the proportion with EC_{50} values of greater than 0.5 who are NRs is 100%.

Average PEG-IFN effectiveness is dependent upon the magnitude and duration that the PEG-IFN concentration is above the EC_{50} (Fig. 3A). The average drug concentration, $\bar{C}$, divided by EC_{50} ($\bar{C}/EC_{50}$) (Table 2, Fig 3B), a quantity we call the therapeutic quotient, and the PEG-IFN concentration at day 7 divided by EC_{50} ($C(7)/EC_{50}$), are significantly increased in responders (SVR vs. NR, $\bar{C}/EC_{50}$: $P = .012$ and $P = .016$ and $C(7)/EC_{50}$: $P = .007$ and $P = .02$; respectively for weeks 1 and 2). During the first 2 weeks of PEG-IFN, a therapeutic quotient ($\bar{C}/EC_{50}$) = 1 has a positive predictive value of 46.2% and a negative predictive value of 100% and $C(7)/$

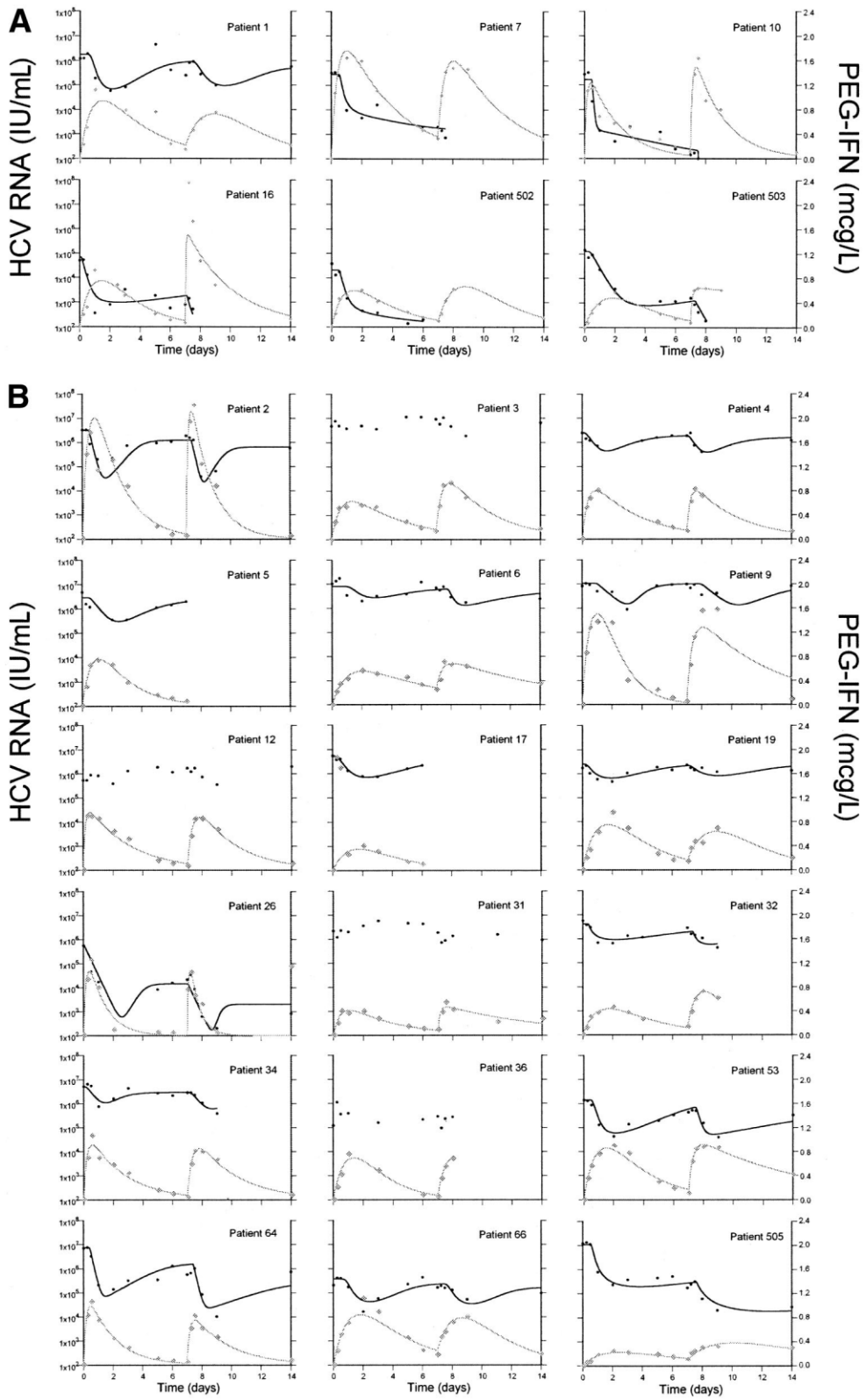

Fig. 2. PEG-IFN serum concentrations and HCV RNA over the first 2 weeks of treatment in (A) SVRs and (B) NRs. Graphs show drug concentration data (♦) and best-fit theoretical curve (gray line) (right axis, μg/L) and viral load data (●) and best-fit curve (black line) (left axis, IU/mL) from our model.

Table 2. Drug Concentration and Effectiveness Calculated From the Estimated Parameters

Patient No.	$\bar{C}1$ (μg/L)	$\bar{C}2$ (μg/L)	$\bar{C}1/EC_{50}$	$\bar{C}2/EC_{50}$	$C(7)/EC_{50}$	$C(14)/EC_{50}$	$\bar{\varepsilon}1$	$\bar{\varepsilon}2$	$\varepsilon 1_{max}$	$\varepsilon 2_{max}$
1*	0.58	0.51	1.3	1.2	0.50	0.53	0.61	0.58	0.95	0.88
7*	0.99	0.93	2.2	2.0	0.79	0.76	0.74	0.73	0.94	0.92
10*	0.43	0.50	29	34	3.6	4.0	0.92	0.94	0.99	0.99
16*	0.41	0.64	26	40	6.2	11	0.94	0.96	0.98	0.99
502*	0.35	0.43	15	18	4.8	6.7	0.91	0.94	0.96	0.97
503*	0.30	0.60	5.1	10	1.9	8.3	0.92	0.98	0.98	0.99
Median	0.42	0.55	10.1	14.0	2.8	5.4	0.92	0.94	0.97	0.98
Interquartile range	0.34	0.23	24.8	33.7	4.4	8.3	0.22	0.27	0.35	0.08
2	0.78	0.54	1.2	0.85	0.13	0.04	0.46	0.31	0.99	0.99
3	0.39	0.49								
4	0.43	0.39	0.97	0.88	0.33	0.26	0.43	0.38	0.86	0.85
6	0.42	0.52	0.87	1.1	0.59	0.73	0.38	0.55	0.64	0.80
9	0.59	0.84	0.92	1.3	0.05	0.70	0.38	0.66	0.97	0.94
12	0.42	0.42								
19	0.47	0.44	1.0	0.99	0.38	0.45	0.48	0.47	0.74	0.67
26	0.25	0.14	4.1	2.2	0.07	0.00	0.55	0.30	1.00	1.00
31	0.23	0.32								
34	0.38	0.41	0.70	0.76	0.14	0.19	0.27	0.31	0.89	0.86
36	0.36	0.48								
53	0.51	0.66	2.8	3.6	0.93	2.34	0.81	0.91	0.96	0.96
64	0.31	0.31	3.6	3.6	0.33	0.71	0.69	0.80	0.99	0.99
66	0.55	0.51	1.0	0.99	0.46	0.37	0.51	0.46	0.87	0.84
505	0.17	0.33	4.6	8.6	3.4	7.6	0.93	0.98	0.97	0.99
Median	0.42	0.44	1.0	1.1	0.33	0.45	0.48	0.47	0.96	0.94
Interquartile range	0.20	0.19	2.7	2.7	0.5	0.5	0.31	0.49	0.13	0.15
P value	NS	NS	.012	.016	.007	.02	.02	.017	NS	NS

Abbreviations: $\bar{C}1$, average first-week PEG-IFN concentration; $\bar{C}2$, average second-week PEG-IFN concentration; $\bar{C}1/EC_{50}$, average first-week concentration divided by EC_{50}; $\bar{C}2/EC_{50}$, average second-week concentration divided by EC_{50}; $C(7)/EC_{50}$, PEG-IFN concentration at day 7 divided by EC_{50}; $C(14)/EC_{50}$, PEG-IFN concentration at day 14 divided by EC_{50}; $\bar{\varepsilon}1$, average effectiveness during week 1; $\bar{\varepsilon}2$, average effectiveness during week 2; $\varepsilon 1_{max}$, maximum effectiveness during week 1; $\varepsilon 2_{max}$, maximum effectiveness during week 2; NS, not significant.

*sustained virological responder.

$EC_{50} = 0.5$ has a positive predictive value of 66.7% and a negative predictive value of 100%.

Race had an interesting effect on pharmacodynamic and viral kinetic parameters. Comparing SVR and NR patients (not including ETR), the fold difference in median EC_{50} is more pronounced in African American than in Caucasian patients (Δ 11.0 vs. 2.3). Among the patients who achieved SVR status, 66% were Caucasian and 33% were African American. Viral kinetic parameters also differed by race; the median δ among Caucasian SVRs and African American SVRs was 0.66 and 0.33, respectively ($P = .16$). If the analysis is restricted to patients in whom HCV RNA never went below the level of assay detection, δ differs significantly between African American and Caucasian patients ($P = .04$).

Next, we compared maximum (ε_{max}) and average ($\bar{\varepsilon}$) PEG-IFN viral effectiveness between SVRs and NRs (Fig. 3C; Table 2). The median weekly average effectiveness is significantly increased in SVR compared with NR for both weeks ($\bar{\varepsilon}_1 = 0.92$ vs. 0.48; $P = .02$ and $\bar{\varepsilon}_2 = 0.94$ vs. 0.47; $P = .017$, respectively). In contrast, the median maximum effectiveness achieved during the week was not significantly different between SVRs and NRs (Table 2).

Lastly, the median value of n is significantly higher in NR compared with SVR patients (Table 3). Because n determines a patient's sensitivity to changes in IFN concentration, it could be clinically important. For example, when the PEG-IFN concentration is half the EC_{50}, the effectiveness is only 0.06 when $n = 4$ but is 0.33 when $n = 1$. This sharp decline in effectiveness may be the reason why patients with a large value of n tend to be NRs.

Discussion

Approximately 55% of HCV-monoinfected and 27% to 40% of HCV/HIV-coinfected patients treated with PEG-IFN and RBV will achieve a sustained virological response.[2-6] To determine which coinfected patients are likely to respond, at least 12 weeks of therapy and a 2-log decline in HCV RNA are required (*i.e.*, early virological response).[4-6] Consequently, all patients—including those who are unlikely to respond—must complete at least 3 months of therapy with high morbidity to justify the full course of treatment. Currently, a primary challenge in HCV management is the identification of factors that differentiate SVRs and NRs, factors that might eventually

Table 3. Parameter Estimates and Corresponding 95% CIs

Patient No.	FD/V_a1 (μg/L)	FD/V_a2 (μg/L)	k_a1 (day^{-1})	k_a2 (day^{-1})	k_e1 (day^{-1})	k_e2 (day^{-1})	δ (day^{-1})	c (day^{-1})	EC_{50} (μg/L)	t_0 (days)	n
1*	1.52	1.52	1.22 (0.89-1.72)	0.53 (0.46-0.58)	0.32 (0.25-0.44)	0.41 (0.38-0.49)	0.14 (0.00-0.31)	9.93	0.44 (0.36-0.56)	0.41	4
7*	2.35 (2.12-2.60)	1.85 (1.62-2.24)	2.38 (1.83-3.01)	2.00 (1.50-2.60)	0.29 (0.24-0.35)	0.28 (0.23-0.37)	0.90 (0.82-0.99)	15.6 (14.6-17.1)	0.46 (0.41-0.50)	0.46	2
10*	1.52	1.69 (1.42-2.54)	6.47 (3.64-50.7)	7.57 (3.24-NDA)	0.49 (0.37-0.68)	0.50 (0.31-0.99)	0.80 (0.78-0.81)	19.9 (19.5-20.4)	0.01 (0.01-0.01)	0.44	1
16*	1.52	1.52	0.95 (0.67-1.35)	24.4 (6.71-NDA)	0.49 (0.41-0.64)	0.31 (0.05-0.42)	0.25 (0.25-0.25)	5.77 (5.74-5.79)	0.02 (0.02-0.02)	0.06	1
502*	1.01 (0.82-1.59)	1.52	1.15 (0.64-1.61)	0.59 (0.50-0.65)	0.37 (0.27-0.63)	0.48 (0.43-0.61)	0.52 (0.41-0.60)	6.71 (6.66-6.75)	0.02 (0.02-0.02)	0.43	1
503*	1.52	0.53 (0.51-0.55)	0.47 (0.38-0.56)	9.46 (8.41-11.3)	0.63 (0.55-0.79)	0.04 (0.02-0.06)	0.50 (0.50-0.50)	2.78 (2.77-2.78)	0.06 (0.06-0.06)	0.23	2
Median	1.52	1.52	1.18	4.78	0.43	0.36	0.51	8.32	0.04	0.42	1.5
Interquartile range	0.33	0.46	2.65	12.62	0.21	0.26	0.60	11.65	0.43	0.26	1.5
2	3.11 (2.64-3.93)	2.64 (2.21-3.61)	2.40 (1.71-3.06)	7.06 (3.47-88.3)	0.55 (0.45-0.71)	0.70 (0.48-1.17)	0.30 (0.17-0.46)	6.35 (3.99-15.2)	0.64 (0.48-0.87)	0.30	4
3	0.90 (0.77-1.15)	1.11 (0.96-1.43)	1.60 (1.07-2.29)	2.47 (1.65-3.31)	0.27 (0.20-0.36)	0.32 (0.25-0.48)	NA	NA	NA	NA	NA
4	1.05 (0.97-1.14)	0.84 (0.76-0.97)	2.67 (2.22-3.11)	4.30 (3.17-5.92)	0.30 (0.28-0.34)	0.32 (0.25-0.38)	0.09 (0.01-0.17)	2.80 (1.77-6.94)	0.44 (0.33-0.58)	0.01	3
6	0.79 (0.67-1.03)	0.55 (0.42-0.78)	1.67 (0.72-1.48)	2.50 (1.23-5.40)	0.17 (0.12-0.26)	0.12 (0.07-0.20)	0.05 (0.00-0.25)	12.2 (1.99-54.1)	0.48 (0.42-0.63)	0.68	4
9	3.35 (2.03-4.47)	1.52	1.23 (0.88-2.54)	2.67 (1.45-6.37)	0.80 (0.42-1.10)	0.19 (0.08-0.34)	0.02 (0.00-0.17)	1.28 (1.10-2.16)	0.64 (0.43-0.84)	0.75	4
12	1.12 (1.01-1.26)	1.16 (1.07-1.26)	7.02 (4.7-12.4)	2.64 (2.26-3.04)	0.34 (0.28-0.42)	0.38 (0.33-0.44)	NA	NA	NA	NA	NA
19	1.52	1.52	0.79 (0.61-1.08)	0.49 (0.38-0.64)	0.41 (0.33-0.50)	0.45 (0.38-0.59)	0.01 (0.00-0.06)	16.5 (8.34-27.5)	0.45 (0.36-0.54)	0.09	2
26	1.52	1.52	5.57 (3.04-27.6)	10.4 (2.96-NDA)	0.87 (0.30-1.07)	1.59 (0.39-1.98)	0.99 (0.84-1.12)	3.03 (2.39-3.56)	0.06 (0.05-0.08)	0.00	4
31	0.58 (0.47-0.74)	0.43 (0.33-0.59)	2.13 (1.38-3.33)	6.27 (2.41-NDA)	0.30 (0.22-0.41)	0.14 (0.07-0.25)	NA	NA	NA	NA	NA
34	1.14 (1.00-1.44)	1.09 (0.99-1.24)	4.97 (2.90-10.5)	2.96 (2.29-3.55)	0.40 (0.29-0.56)	0.36 (0.29-0.47)	0.30 (0.06-0.64)	2.10 (1.08-4.59)	0.54 (0.39-0.73)	0.00	4
36	1.52	1.52	0.91 (0.76-1.07)	1.04 (1.03-1.05)	0.56 (0.49-0.68)	0.72 (0.70-0.73)	NA	NA	NA	NA	NA
53	1.52	0.94	1.08 (0.93-1.27)	3.31 (2.81-3.86)	0.37 (0.33-0.42)	0.14 (0.12-0.16)	0.01 (0.01-0.01)	7.24 (7.09-7.54)	0.18 (0.17-0.18)	0.43	2
64	1.26 (1.14-1.42)	0.87 (0.75-1.08)	5.37 (4.21-7.45)	5.17 (3.33-8.81)	0.56 (0.46-0.66)	0.40 (0.28-0.60)	0.30 (0.30-0.31)	6.50 (6.41-6.60)	0.09 (0.09-0.09)	0.36	2
66	1.52	1.52	0.86 (0.71-1.10)	0.64 (0.55-0.71)	0.33 (0.28-0.38)	0.43 (0.39-0.52)	0.12 (0.06-0.18)	23.1 (7.54-33.7)	0.52 (0.44-0.57)	0.45	4
505	1.52	1.52	0.18 (0.15-0.20)	0.15 (0.12-0.19)	0.78 (0.70-0.91)	0.48 (0.40-0.58)	0.17 (0.16-0.17)	26.4 (26.1-26.6)	0.04 (0.03-0.04)	0.48	2
Median	1.52	1.16	1.67	2.67	0.40	0.38	0.12	6.50	0.45	0.36	4.0
Interquartile range	0.47	0.65	4.06	4.13	0.26	0.29	0.28	13.70	0.45	0.47	2.0
P value	NS	NS	NS	NS	NS	NS	.044	NS	.014	NS	.025

NOTE. Because the estimates are presented up to 2 decimal places only, the upper and lower bounds may appear to overlap with each other. CIs are presented in parentheses.

Abbreviations: FD/V_a1, (bioavailability × dose / volume of distribution) during first week; FD/V_a2, (bioavailability × dose / volume of distribution) during the second week; k_a1, absorption rate during first week; k_a2, absorption rate during second week; k_e1, elimination rate during first week; k_e2, elimination rate during second week; δ, loss rate of infected cells; c, clearance rate free virus; EC_{50}, effective PEG-IFN concentration that results in 50% decrease in HCV RNA production; t_0, time delay from initiation of IFN until decline in HCV RNA, n, Hill coefficient; NA, not available (because we could not fit these data); NS, not significant.

*sustained virological responder.

be used as biomarkers predictive of a successful treatment outcome. In this study, we asked whether PEG-IFN pharmacodynamics or pharmacokinetics might differentiate SVRs and NRs. Because SVR percentages are decreased in coinfected compared with monoinfected patients, we decided to evaluate these parameters in this difficult-to-treat population. We found that pharmacodynamic measurements, namely EC_{50}, the therapeutic quotient (*i.e.*, the ratio between the average PEG-IFN concentration and ($\bar{C}/EC_{50}$)), and the PEG-IFN concentration on day 7 divided by EC_{50} ($C(7)/EC_{50}$) are significantly different in coinfected SVRs compared with NRs. Prospective studies with larger numbers of patients are needed to evaluate whether these parameters could be used to identify NR patients. $C(7)/EC_{50}$ may be the most clinically meaningful, because it may require fewer measurements over the first week.

Recent data have shown that anti-HCV therapy can be discontinued in patients who do not achieve an early virological response, defined as a minimum 2-log decline in HCV RNA by week 12.[23] Patients who do not achieve an early virological response do not become SVRs. Although pharmacodynamic parameters are derived from analyses of HCV RNA and PEG-IFN kinetics, these parameters may be more sensitive to differences between SVRs and NRs than HCV RNA decline alone. Consequently, these parameters might be used clinically to identify likely SVRs earlier than 12 weeks.

Our estimated pharmacokinetic parameters for PEG-IFN α-2b are equivalent to those published previ-

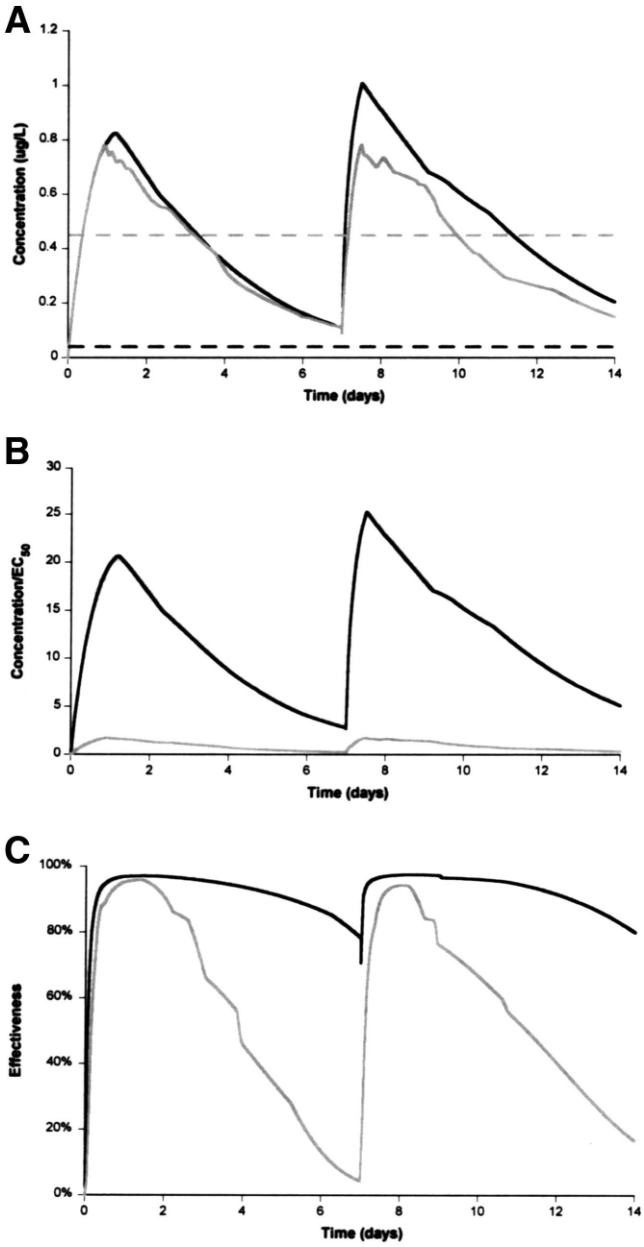

Fig. 3. Median drug concentration and effectiveness. (A) Median drug concentration for SVRs (black line) and NRs (gray line) over the first 2 weeks of treatment. The dashed lines indicate the corresponding estimated median EC_{50}. The drug concentration for SVRs is always above the corresponding EC_{50}. (B) Median therapeutic quotient (*i.e.*, the average PEG-IFN concentration divided by EC_{50}), and (C) median drug effectiveness are significantly higher for SVRs (black line) than for NRs (gray line).

ously,[22,24] suggesting that coinfection does not influence PEG-IFN pharmacokinetics. Serum PEG-IFN concentration (measured as maximum concentration during the week or area under the curve) *per se* was not a factor differentiating SVR from NR, neither were the kinetic parameters of drug absorption and elimination. Rather, we found that the therapeutic quotient and the PEG-IFN concentration at day 7 divided by EC_{50} were significantly increased in both coinfected ETRs and SVRs compared with NRs. These findings suggest that responders are patients with sufficient "effective" PEG-IFN to control viral replication during the dosing interval. These conclusions are corroborated by the finding that EC_{50} was decreased in coinfected SVRs compared with NRs, suggesting that responders might require lower serum PEG-IFN concentrations for successful treatment outcomes. To our knowledge, however, similar pharmacodynamic data have not been reported in HCV-monoinfected patients.

PEG-IFN effectiveness varied due to the changes in drug concentration (Fig. 3). Average effectiveness was significantly increased in coinfected responders compared with nonresponders during both the first and second weeks. Increased effectiveness in SVR is consistent with previous studies using both standard IFN[25,26] and PEG-IFN α.[27-29] However, it is difficult to quantitatively compare our estimates of PEG-IFN effectiveness with those of previous studies, which used simpler models that did not account for changes in serum PEG-IFN α concentration.

What factors might account for increased effectiveness of PEG-IFN α-2b in SVRs compared with NRs? In our model, effectiveness depends on drug concentration, the Hill coefficient, and EC_{50}. The first variable does not differ significantly between responders and nonresponders, whereas n and EC_{50} are lower in SVRs. The PEG-IFN concentration that results in a 50% reduction in HCV RNA during the first phase, is lower in SVRs. Both viral and host factors may play a role in determining EC_{50}. The effectiveness of standard IFN is higher in genotype 2–infected patients than in those with genotype 1 infection.[30] Even within a single genotype, sequence differences in NS5A may determine IFN sensitivity.[31,32] Host factors have also been shown to play a role, as exemplified by the difference in IFN effectiveness observed in Caucasian and African American patients infected with HCV genotype 1.[33-35] Given the complexity of IFN responsiveness, it is not surprising that we did not observe a threshold EC_{50}, a threshold therapeutic quotient, or a $C(7)/EC_{50}$ that correlate with sustained virological response. A threshold effect could be masked by the relatively small sample size of this study or the variation in serum drug concentration among patients.

Our analysis only considered the effect of PEG-IFN concentration on HCV RNA. Differences in RBV concentration may also account for differences in viral response. We have recently shown that in patients with low IFN effectiveness, the effects of RBV may be important in determining second-phase slope and, hence, whether patients achieve a sustained virological response.[36] However, RBV does not appear to influence first-phase slope

(*i.e.*, estimates of IFN effectiveness).[36,37] For the analysis of IFN effectiveness, the kinetic analysis used here may also be applicable to other PEG-IFN formulations (*i.e.* PEG-IFN α-2a), as HCV RNA rebounds at the end of the weekly dosing interval have been noted in some patients treated with PEG-IFN α-2a.[28,38]

In conclusion, we used an HCV dynamic model that incorporates PEG-IFN α-2b phamacodynamic and pharmacokinetic parameters to account for the waning effectiveness of drug at the end of the weekly dosing interval as well as a potential rebound in HCV RNA. We found that a key parameter in determining sustained virological response in HIV/HCV–coinfected patients is the therapeutic quotient, the weekly average PEG-IFN concentration divided by the EC_{50}, a ratio that is significantly higher in responders compared with nonresponders. Currently, the NR segment of the HCV-infected population continues to expand, and new treatments for HCV are not likely to be widely available for several years, particularly in coinfected patients. The EC_{50}, $\bar{C}/EC_{50}$, the therapeutic quotient, or the PEG-IFN concentration on day 7 divided by EC_{50} may be useful predictors of a successful response to treatment. These parameters should be tested prospectively in monoinfected and coinfected patients. If patients who are likely to respond could be identified earlier than 12 weeks after initiation of treatment, therapy could be limited to those likely to achieve a sustained virological response.

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