ORIGINAL RESEARCH ARTICLE

Drugs 2008; 68 (6): 791-801
0012-6667/08/0006-0791/$53.45/0

Early and Sustained Virological Response in Non-Responders with Chronic Hepatitis C

A Randomized Open-Label Study of Pegylated Interferon-α-2a versus Pegylated Interferon-α-2b

Gaetano Scotto,[1] *Vincenzina Fazio*,[2] *Chiara Fornabaio*,[1] *Alessandra Tartaglia*,[1] *Rocco Di Tullio*,[1] *Annalisa Saracino*[1] and *Gioacchino Angarano*[1]

1 Clinic of Infectious Diseases, University of Foggia, Foggia, Italy

2 Clinical Chemistry Laboratory, Hospital of Foggia, Foggia, Italy

Abstract

Objectives: The purpose of this randomized open-label study was to assess the efficacy of treatment with pegylated interferon-α-2a versus pegylated interferon-α-2b, both plus ribavirin, in inducing early and sustained virological response (EVR and SVR) in chronic hepatitis C non-responders.

Patients and methods: A total of 108 patients with chronic hepatitis C who were non-responders to previous combined therapy (standard interferon-α plus ribavirin for ≥3 months) were enrolled and equally randomized into two groups in this intention-to-treat analysis. The patients exhibited similar baseline features. One group received subcutaneous pegylated interferon-α-2a 180 μg once weekly, while the other was treated with subcutaneous pegylated interferon-α-2b 1.5 μg/kg once weekly. Ribavirin 15 mg/kg/day was included in both protocols. Treatment duration for EVR was 12 weeks. Patients who demonstrated non-detectable hepatitis C virus (HCV) RNA or a ≥2 $\log_{10}$ reduction in viral load at week 12 continued therapy up to 48 weeks, with assessments every 3 months during a follow-up of 24 weeks.

Results: All patients in both groups completed the EVR study, then seven patients receiving pegylated interferon-α-2a and seven patients receiving pegylated interferon-α2b discontinued treatment as a result of severe adverse effects. After 12 weeks of treatment, viral load reduction was >2 $\log_{10}$ with both pegylated interferon-α-2a (−2.53) and pegylated interferon-α-2b (−2.48) with no significant difference. At the end of week 48, HCV RNA was undetectable in 14 of 54 patients (25.9%) receiving pegylated interferon-α-2a and in 15 of 54 patients (27.7%) receiving pegylated interferon-α-2b. When terminating follow-up, an SVR was observed in 11 of 54 patients (20.4%) who received pegylated interferon-α-2a and 10 of 54 patients (18.4%) receiving pegylated interferon-α-2b. The incidence and severity of adverse events was similar in both groups.

Conclusions: Our results seem to show that in chronic hepatitis C patients who are non-responsive to previous therapy, EVR to the two pegylated interferons did not significantly differ with a similar therapeutic efficacy defined as SVR.

In patients with hepatitis C virus (HCV)-related chronic hepatitis, antiviral therapy has furnished either very poor (standard interferon-α ± ribavirin)[1-4] or sub-optimal (pegylated interferon-α ± ribavirin) results, in terms of a sustained virological response (SVR).[5-7] At present, there is no suitable therapy capable of inducing SVR in all patients; therefore, a definite milestone would be the identification of predictive factors for complete response to antiviral treatment.[8] Early virological response (EVR), defined as non-detectable HCV RNA levels at the 12th week of treatment, with a distinctive biphasic reduction, is one of the most important predictive factors for SVR when using both standard and pegylated interferon-α.

Mathematical analysis of viral kinetics has demonstrated the existence of an equilibrium between viral production and clearance related to the rapid turnover of these virions (short half-life of approximately 3 hours), and the high index of HCV production (10^{10}–10^{12}/day). Antiviral therapy can significantly modify this equilibrium with the following two-phase HCV RNA decline:[9] (i) a first, rapid decrease at 24–48 hours due to a specific interferon's antiviral action with a halt in viral production and release of intracellular virions; (ii) a second slower phase starting on day 3 related to the gradual elimination of the infected liver cells and a lower level of production of newly HCV-infected liver cells.[10,11]

While the first phase may be influenced by the viral genotype and the treatment regimen (in fact, high doses of standard or pegylated interferon caused a remarkable inhibition of HCV production [>90%]), it shows little correlation with long-term outcomes. However, the second phase, which is less influenced by interferon dosage and genotype, is a better marker of SVR (depending on whether the response is rapid, slow or flat).[12-14]

The combination of standard interferon and ribavirin does not seem to influence these two viral kinetic phases.[15] Patients who are non-responders to previous standard interferon plus ribavirin are a difficult-to-treat group (particularly those with HCV genotype 1). However, this group can, in some cases, be successfully retreated with pegylated interferon plus ribavirin as a result of the improved pharmacokinetic and pharmacodynamic characteristics of the pegylated derivatives compared with standard interferons.

The two pegylated interferons currently available, in association with ribavirin, are considered the most effective therapy for chronic hepatitis C, but demonstrate totally different chemico-physical features related to the pegylation system (α-2a is spiraliform, α-2b is linear) and to the different molecular weights (α-2a is 40 kD, α-2b is 12 kD).[16,17] The diversity of the two molecules determines different pharmacokinetic properties: a longer half-life and a smaller distribution volume for α-2a, and different routes of elimination (α-2a mainly through the liver and α-2b through the kidney).[18,19] However, it is important to verify if these structural and pharmacokinetic differences can influence viral kinetics. Previously published studies have assessed the viral load pattern under therapeutic pressure during the first 3 months of treatment, with similar results, but only in a few cases and only in treatment-naive patients was a direct comparison made between the two molecules yielding discordant results (see discussion section for details).[20,21] In addition, results for previously-treated patients were obtained in only one study.[22]

The purpose of this study was to assess if the diverse pharmacokinetic properties of the two pegylated interferons could influence viral kinetics and EVR, and hence provide variable percentages of SVR, in a cohort of patients who were non-respond-

ers to a previous course of standard interferon plus ribavirin therapy for a period of ≥3 months.

Patients and Methods

Criteria for Inclusion/Exclusion of Patients

Our intention-to-treat study included adult patients with well compensated chronic HCV-related hepatitis, non-responding to previous antiviral treatment (interferon plus ribavirin for a period of at least 3 months) who met the following requirements: (i) serum ALT levels twice the upper limit of normal on at least three occasions during the 6 months before screening; (ii) serum HCV RNA levels that could be estimated and quantified by a real-time polymerase chain reaction (PCR) [sensitivity <100 IU/mL]; (iii) liver biopsy performed within 1 year before enrollment indicating histological diagnosis of chronic hepatitis according to Knodell's classification; and (iv) no antiviral and/or immunosuppressive treatment during the 6 months before enrollment.

For inclusion in the study, the following parameters were required: (i) haemoglobin level ≥13 g/dL for males and ≥12 g/dL for females; (ii) leukocyte count ≥4000 mm^3; (iii) neutrophil count ≥1800 mm^3; (iv) platelet count ≥100 000 mm^3; (v) and α-fetoprotein levels <50 μg/mL. An ultrasonography procedure performed within 3 months before the screening had to exclude the presence of hepatocellular carcinoma.

Subjects with liver disease related to other factors (e.g. hepatitis B virus infection, Wilson's disease, α-1-antitrypsin deficiency, haemochromatosis, autoimmune hepatitis, iatrogenic and dysmetabolic forms) were excluded from the study. Moreover, HIV-infected patients, active drug abusers and patients with pre-existing clinical (e.g. epilepsy, severe depression) and/or social conditions were also excluded. In addition, it was deemed appropriate to exclude patients who, although currently compensated, had previously experienced episodes of liver decompensation (e.g. ascites, bleeding from oesophageal varices, encephalopathy). Breast feeding women were excluded and a negative pregnancy test was required prior to study enrollment. Patients of both genders were requested to adopt proper contraceptive measures during the entire study period.

Study Design and Organization

The study was performed between November 2001 and December 2005 at the Clinic of Infectious Diseases, University of Foggia, Italy. The local ethics committee reviewed and approved the scientific background, the rationale and the methods used in the study. A written informed consent, containing information about the trial (doses, period of therapy, follow-up) and the goal of the study, was obtained from each subject enrolled. All study procedures were in agreement with the Helsinki Declaration.

A complete medical history was obtained for all patients, in particular regarding liver disease. An evaluation of psychiatric conditions, a chest radiograph, an ECG and a liver biopsy (unless performed previously) were carried out. All histological examinations were assessed by a single blinded pathologist. The laboratory tests (haematological parameters, hepatocellular necrosis indexes, α-fetoprotein, qualitative and/or quantitative serum HCV RNA by means of real-time PCR [HCV rotor gene RT-PCR reagent, Qiagen, Milan, Italy; cut-off <100 IU/mL]) were performed in the same diagnostic centre throughout the study. HCV genotype (Inno-Lipa HCV II kit, Immunogenetics, Zwijmarden, Belgium) according to Simmonds' classification was identified in all patients.

Patients were enrolled by a pre-established numerical randomization method determined by the Clinic's medical staff. The group assignment was non-blinded. Pegylated interferon-α-2a 180 μg and pegylated interferon-α-2b 1.5 μg/kg were administered subcutaneously once weekly. In all patients, interferon therapy was combined with ribavirin at a dose of 15 mg/kg/day orally. The therapy was self-administered by each patient and compliance was assessed by periodical (monthly) counselling. All the patients who demonstrated undetectable HCV RNA levels after 12 weeks of treatment continued treatment to 48 weeks. Patients with detectable HCV RNA levels after 12 weeks of treatment, but

with a ≥2 $\log_{10}$ viral load decrease compared with baseline values, continued therapy up to 24 weeks. When persistently high levels of HCV RNA were noted, treatment was discontinued, whereas if HCV RNA could no longer be detected, therapy was continued up to 48 weeks. Lastly, patients who did not exhibit a >2 $\log_{10}$ reduction of HCV RNA levels after 12 weeks discontinued therapy.

During the entire study period (48 weeks of treatment and 24 weeks follow-up after stopping therapy), the patients were monitored by assessing vital signs, bodyweight, adverse effects, treatment compliance, haematological and biochemical parameters, and HCV RNA serum levels.

To compare the efficacy of the two pegylated interferons, the following parameters were evaluated: bodyweight, genotype and viral load.

Clinical and laboratory examinations were performed monthly during treatment and every 3 months during follow-up. In addition, complete blood chemistry tests and glucose-6-phosphate-dehydrogenase levels were evaluated weekly during the first month of treatment after the administration of ribavirin. Serum HCV RNA levels were assessed at the beginning of therapy and every 3 months during the treatment period (for evaluation of SVR) and follow-up.

Evaluation of Early Virological Response (EVR)

To establish the best definition of EVR, during the first 12 weeks of treatment, the viral load was evaluated at baseline and after 24, 48 and 72 hours on days 7, 14, 21 and 28, and at the end of week 12. Patients were then classified according to the following four response patterns:

1. Non-responders: patients who showed no decrease in the viral load versus baseline values.
2. Flat partial responders: patients who, after a rapid first-phase decrease of 1–2 $\log_{10}$ (within 1–2 days), did not demonstrate a further decrease in viral load.
3. Slow partial responders: patients who, after a rapid first-phase decrease, demonstrated a second phase with a markedly gradual HCV RNA decrease, which could be estimated at week 12 ($\leq 10^5$ IU/mL).
4. Rapid virological responders: patients with rapid first and second stages of viral load response, and early non-assessable HCV RNA (within 4 weeks from starting treatment).

In our study, the main purpose of this definition was to compare EVR of patients treated with the two pegylated interferons. Moreover, the evaluation of EVR was the necessary endpoint to exclude patients with a high probability of therapeutic failure from treatment continuation.

Efficacy Assessment of Sustained Virological Response (SVR)

Treatment response was defined as follows:

1. Primary endpoint: a SVR defined as undetectable serum HCV RNA for 24 weeks after the end of treatment.
2. Secondary endpoint: a sustained biochemical response defined as normalization of ALT levels 24 weeks after the end of treatment.

Treatment efficacy was assessed in the enrolled subjects after at least one drug administration.

Assessment of Adverse Effects

Adverse effects were monitored by means of clinical and laboratory evaluations. Data concerning adverse events included the severity, incidence and duration of each event. Adverse effects were classified according to WHO guidelines as follows: (i) mild; (ii) moderate; (iii) severe; and (iv) life threatening. In the case of life-threatening adverse events, treatment was definitively discontinued and the patient was monitored for 24 weeks. When severe adverse effects were noted, the antiviral dose was reduced and eventually increased again following improvement of the clinical and/or laboratory parameters observed.

Statistical Analysis

The viral load was defined as the base 10 log. EVR was estimated as the $\log_{10}$ difference between the viral load baseline value and the serum levels obtained at the various timepoints using the ANOVA test between-with a two-factor mixed de-

sign. To compare the different EVR percentages in this intention-to-treat analysis, the Student's t-test and the Mann-Whitney method were used in the two patient groups for quantitative evaluation of the qualitative and quantitative variables. The chi-square test was used to compare the different response rates between the two groups of patients. A p-value of <0.05 was considered statistically significant.

Results

A total of 108 patients (62 men and 46 women, average age 45.2 years) meeting the inclusion requirements were enrolled into the study. The demographic and clinical characteristics of both groups of patients were similar (see table I). Among the 54 patients enrolled in each treatment group, seven patients receiving pegylated interferon-α-2a and seven patients receiving pegylated interferon-α-2b discontinued treatment as a result of severe adverse effects. Furthermore, nine patients (six receiving pegylated interferon-α-2a and three receiving pegylated interferon-α-2b) spontaneously withdrew from therapy (see figure 1).

Table I. Baseline characteristics of patients enrolled in the study

Characteristics	Pegylated interferon-α-2a	Pegylated interferon-α-2b
Mean age (y)	45.65 ± 9.62	44.74 ± 9.31
Sex (M/F)	33/21	29/25
Mean bodyweight (kg)	80.3	79.6
No. of patients <75 kg	36	34
No. of patients >75 kg	18	20
HCV genotype		
1	37	40
2	5	3
3	3	3
4	9	8
HVC RNA (normal value <100 IU/mL)	$2.2 \pm 3 \times 10^6$	$1.9 \pm 5 \times 10^6$
Cause of infection		
blood transfusion	11	9
intravenous drug use	26	30
community acquired	17	15
Mean Knodell index	10 ± 1	9 ± 4
Patients affected by cirrhosis	9	10
ALT (IU/mL)[a]	195 ±21	189 ±33
Previous therapy	Standard interferon + ribavirin	Standard interferon + ribavirin
Duration of previous treatment (wk)	12	12

a ALT normal value <40 IU/mL.

HCV = hepatitis C virus.

Viral Kinetics

The baseline viral load was very similar in the two groups: 6.07 $\log_{10}$ in the group treated with pegylated interferon-α-2a and 6.04 $\log_{10}$ in the group receiving pegylated interferon-α-2b (p-value not significant); serum HCV RNA levels were >800.000 UI/mL in 46% of those patients receiving pegylated interferon-α-2a versus 43% of those receiving pegylated interferon-α-2b. The results of viral kinetic evaluations were analysed according to the different timepoints, as shown in table II.

In the first 72 hours of treatment, the viral load reduction did not significantly differ between the two patient groups. At the 24-hour timepoint, 13 pegylated interferon-α-2a recipients and 14 pegylated interferon-α-2b recipients demonstrated a reduction of viral load of ≥1 $\log_{10}$.

After the first week of treatment, a lower viral load was noted in both groups compared with baseline values. At the end of week 4, 28.7% of patients taking pegylated interferon-α-2a and 29.0% of patients taking pegylated interferon-α-2b demonstrated a ≥3 $\log_{10}$ viral load reduction. After 12 weeks, a further reduction of HCV RNA serum levels was observed in both groups.

The EVR pattern was similar in patients treated with pegylated interferon-α-2a and those treated with pegylated interferon-α-2b (−2.53 $\log_{10}$ vs −2.48 $\log_{10}$; p = 0.94) [table II].

HCV RNA was no longer detectable in 15 of 54 patients (27.8%) receiving pegylated interferon-α-2a and in 17 of 54 patients (31.5%) receiving pegylated interferon-α-2b at week 12.

Genotype and Rate of EVR in the Two Patient Groups

The distribution of patients according to genotype and different degrees of EVR, in relation to genotype and group are shown in table III.

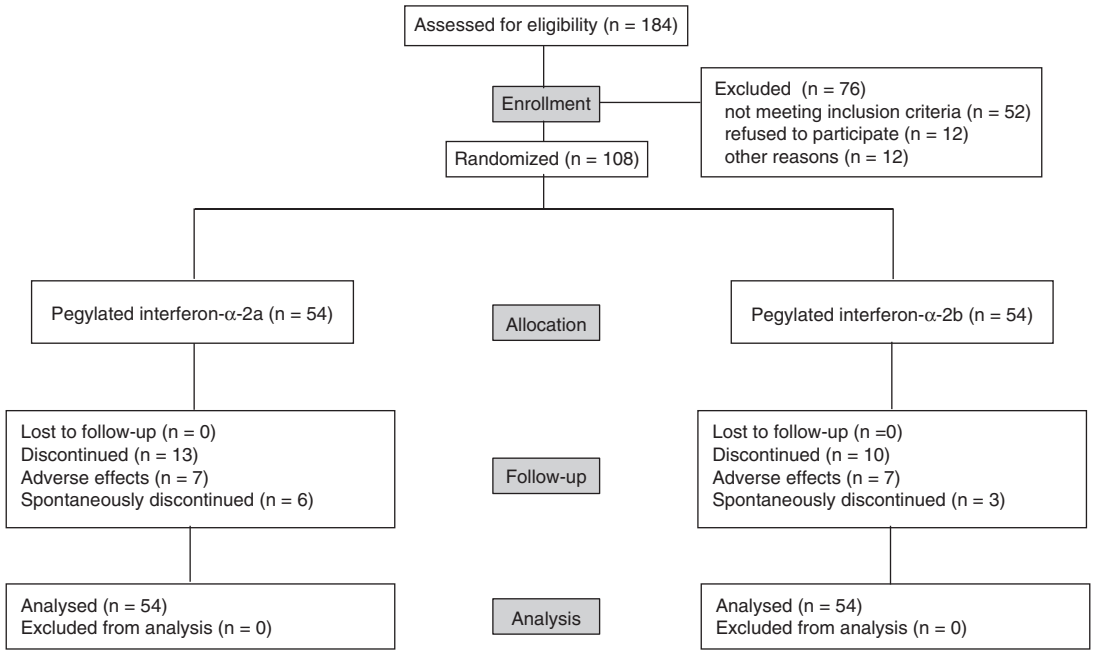

Fig. 1. Patient disposition.

EVR and SVR

In an intention-to-treat analysis, at the end of the 24 weeks follow-up, a SVR was found in 11 of 54 patients (20.4%) receiving pegylated interferon-α-2a and 10 of 54 patients (18.5%) receiving pegylated interferon-α-2b (p-value not significant). Prevalence of the different degrees of EVR and SVR in the two groups is shown in table IV. Analysing this response in relationship to the EVR, the following data were found:

1. In patients who at 24 hours exhibited a ≤1 $\log_{10}$ viral load reduction, an SVR was obtained in only 3 of 41 patients (7.3%) receiving pegylated interferon-α-2a and 3 of 40 patients (7.5%) pegylated interferon-α-2b (p-value not significant).
2. The reduction of viral load ≥1 $\log_{10}$ in the first week of therapy was significantly higher in patients with SVR. Analysing the two cohorts of subjects, we found that 9 of 11 patients (81.8%) with SVR treated with pegylated interferon-α-2a and 7 of 10 patients (70.0%) with SVR in therapy with pegylated interferon-α-2b exhibited a ≥1 $\log_{10}$ reduction of HCV RNA versus the baseline values.
3. An SVR was obtained in 11 of 15 patients (73.3%) receiving pegylated interferon-α-2a and in 10 of 17 patients (58.8%) pegylated interferon-α-2b in whom HCV RNA levels were no longer assessable at 3 months.
4. In all the subjects with SVR, whether treated with pegylated interferon-α-2a or pegylated interferon-α-2b, a decline of viral load ≥2 $\log_{10}$ was obtained.

Factors Related to the Virological Response

When comparing baseline demographic and clinical characteristics of patients treated with the two pegylated interferons, the following data was observed with regard to SVR:

1. The bodyweight of 7 of 11 patients (63.6%) with SVR receiving pegylated interferon-α-2a was <75 kg, while 8 of 10 responder patients (80%) receiving pegylated interferon-α-2b weighed >75 kg (p-value not significant).
2. Genotype and viral load: 4 of 11 responder patients (36.4%) who received pegylated interferon-α-2a were classified as genotype 1, four as genotype 2, two as genotype 3 and one as genotype 4. Among

pegylated interferon-α-2b recipients with SVR, four of ten (40%) were genotype 1, three were genotype 2, two were genotype 3 and one was genotype 4. In genotype 1 subjects who received pegylated interferon-α-2a, 4 of 37 patients (10.8%) had SVR with a mean basal viral load of 1.9×10^6 IU/mL, while in those receiving pegylated interferon-α-2b, the response rate was 10.0% (4 of 40) with a mean viral load of 1.3×10^6 IU/mL (p-value not significant) [table III].

Biochemical Response

At the end of treatment, a normalization of the cell necrosis index was observed in 16 of 54 patients (29.6%) who received pegylated interferon-α-2a and in 17 of 54 patients (31.5%) who received pegylated interferon-α-2b.

During the follow-up period, four patients from each group relapsed with the return of ALT levels to pathological values. Therefore, when terminating the study (72 weeks), normal ALT levels were observed in 12 of 54 (22.2%) pegylated interferon-α-2a recipients and 13 of 54 (24.1%) pegylated interferon-α-2b recipients (p-value not significant).

Adverse Effects

The incidence and severity of adverse effects were similar for both pegylated interferon-α-2a and α-2b. Severe psychiatric disorders (e.g. manic syndrome, hallucinations, suicidal thoughts), resulting in eventual discontinuation of therapy were observed for six patients (three patients in each arm). Treatment discontinuation was also necessary for three α-2a recipients and two α-2b recipients as a result of marked weight loss (>15% of bodyweight). Two patients, one in each study arm, discontinued therapy because of severe headaches and another patient (receiving interferon-α-2b) as a result of continuous fever (≈39°C); both of these adverse events were observed for more than 2 weeks. The main adverse events are described in table V.

Six pegylated interferon-α-2a recipients and three pegylated interferon-α-2b recipients with leukopenia and/or thrombocytopenia required a dose reduction (α-2a was reduced to 135 μg/week and α-2b was reduced to 1 μg/kg/week). Five pegylated interferon-α-2a and three pegylated interferon-α-2b recipients required a dose reduction of ribavirin to the effective dose of 10.6 mg/kg/day. No pathological alterations in creatinine and uric acid serum levels were observed, but in all subjects, a moderate ribavirin-dependent hyperbilirubinaemia, due to haemolytic anaemia, was observed.

Discussion

Recent studies have demonstrated that an early reduction of HCV RNA serum levels during the course of therapy with standard interferons may function as a marker of response and therefore would be extremely useful in determining therapeu-

Table II. Viral kinetics in the two treatment groups enrolled in the study

Time from start of therapy	Pegylated interferon-α-2a (log_{10} IU/mL)		Pegylated interferon-α-2b (log_{10} IU/mL)		p-Value
	mean HCV RNA ± SD	mean change from baseline	mean HCV-RNA ± SD	mean change from baseline	
Baseline	6.07 ± 0.76		6.04 ± 0.83		0.84[a]
24 h	5.79 ± 0.81	−0.28	5.79 ± 0.77	−0.25	1.00[a]
48 h	5.67 ± 0.79	−0.40	5.67 ± 0.79	−0.37	1.00[a]
72 h	5.67 ± 1.11	−0.40	5.45 ± 0.94	−0.59	0.85[a]
1 wk	4.88 ± 1.32	−1.19	4.96 ± 1.37	−1.08	0.75[a]
2 wk	4.51 ± 1.46	−1.56	4.67 ± 1.44	−1.37	0.56[a]
3 wk	4.36 ± 1.52	−1.71	4.51 ± 1.21	−1.53	0.57[a]
4 wk	3.95 ± 1.67	−2.12	4.04 ± 1.66	−2.00	0.78[a]
3 mo	3.54 ± 1.61	−2.53	3.56 ± 1.48	−2.48	0.94[a]

a Not significant.

HCV = hepatitis C virus.

Table III. Patients' (pts) distribution according to genotype and rate of early virological response (EVR) and sustained virological response (SVR)

Response	Pegylated interferon-α-2a				Pegylated interferon-α-2b			
	Gen 1	Gen 2	Gen 3	Gen 4	Gen 1	Gen 2	Gen 3	Gen 4
Baseline HCV-RNA (log_{10} IU/mL)	6.28 ± 0.86	5.87 ± 0.68	6.03 ± 0.64	6.09 ± 0.83	6.11 ± 0.78	6.02 ± 0.88	5.95 ± 0.91	6.10 ± 0.75
EVR (no. of pts)	37	5	3	9	40	3	3	8
non responders [no. of pts] (%)	31 (83.8)	0	1 (33.3)	4 (44.5)	32 (80)	0	0	2 (25)
flat partial responders [no. of pts] (%)	2 (5.4)	0	0	1 (11.1)	1 (2.5)	0	0	2 (25)
slow partial responders [no. of pts] (%)	1 (2.7)	1 (20)	0	2 (22.2)	3 (7.5)	1 (33.3)	2 (66.7)	2 (25)
rapid virological responders [no. of pts] (%)	3 (8.1)	4 (80)	2 (66.7)	2 (22.2)	4 (10)	2 (66.7)	1 (33.3)	2 (25)
SVR [no. of pts] (%)	4 (10.8)	4 (80)	2 (66.7)	1 (11.1)	4 (10)	3 (100)	2 (66.7)	1 (12.5)

Gen = genotype; **HCV** = hepatitis C virus.

tic decisions.[9,11] EVR has been defined as non-detectable HCV RNA levels at the 12th week of treatment and appears with a distinctive biphasic viral load reduction.[10,11] The importance of EVR as a prognostic factor has recently been noted for patients treated with pegylated interferons.[13,23,24] In a comparative study evaluating the serum concentrations at 24, 48, 120 and 168 hours after administration of pegylated interferon-α-2a 180 μg or pegylated interferon-α-2b 1 μg/kg, it was found that the former had a higher serum concentration. Indeed, in 58% and 92% of patients treated with pegylated interferon-α-2b, the drug was no longer assessable at 120 and 168 hours, compared with the subjects treated with pegylated interferon-α-2a, in whom the serum concentration was still therapeutically effective.[8] The difference in the pharmacokinetic properties of the pegylated interferons would seem to have an impact on viral kinetics, as viral suppression is the expression of therapeutically effective drug serum concentrations.

Two studies compared the viral kinetics in treatment-naive patients treated with pegylated interferon-α-2a or pegylated interferon-α-2b.[20,21] In a pilot study directly comparing the two molecules and their efficacy to induce EVR in a small cohort of treatment-naive patients, Bruno et al.[20] showed that, although the decline in viral load in the two groups was similar during the first 4 weeks of treatment, pegylated interferon-α-2a induced a more significant viral clearance after 12 weeks of treatment, thus reflecting the different serum concentrations of the two molecules when administered once weekly. In the second comparative study,[21] 36 patients received pegylated interferon-α-2a or -α-2b as monotherapy for the first 4 weeks, followed by a combi-

Table IV. Early virological response (EVR) and sustained virological response (SVR) in the two treatment groups[a]

Type of EVR	Pegylated interferon-α-2a [no. of pts] (%)		Pegylated interferon-α-2b [no. of pts] (%)	
	EVR	SVR	EVR	SVR
Non responders	36 (66.7)	0	34 (63)	0
Flat partial responders	3 (5.5)	0	3 (5.5)	0
Slow partial responders	4 (7.4)	2 (3.7)	8 (14.8)	3 (5.5)
Rapid responders	11 (20.4)	9 (16.7)	9 (16.7)	7 (12.9)

a p-Values not significant.

pts = patients.

nation with ribavirin for a further 4 weeks. The results showed that pegylated interferon-α-2b provoked a significantly greater viral load reduction during weeks 1 and 4, and analysis of these data evidenced that this reduction in viral load was enhanced at the end of week 8. The results of these studies comparing the viral kinetics of both pegylated interferons are discordant. A structural difference between pegylated interferons exists, but the possibility that this difference truly induces a diverse EVR remains still unanswered.

Our study, which, at present, appears to be the only one considering non-responders to previous combined treatments, aimed to assess the viral kinetic pattern in this class of patients, and to compare the EVR and SVR induced by the two pegylated interferons. In our experience, the initial EVR stage (24 hours) was lower than that found in previous studies in treatment-naive patients treated with one of the pegylated interferons.[13,20,23,24] In other reports on non-responder subjects retreated with standard interferon, no differences were found between the EVR in treatment-naive patients and in non-responders.[25,26] On the contrary, in other studies, a single dose of interferon in non-responder patients was found to induce a lower EVR compared with that observed in treatment-naive patients; however, the reduction in viral load was not uniform in all non-responder patients, but was a function of the degree of response to previous therapy.[27,28]

We observed that, after 24 hours, a high percentage of patients from both groups did not show a ≥1 $\log_{10}$ HCV RNA reduction. This finding might be used as a predictive factor of therapeutic failure in ≈90% of patients with this response. The successive timepoint at 48–72 hours initially showed a progressive reduction of viral load in both patient groups, followed by stabilization in patients who received pegylated interferon-α-2a, whereas a moderate decrease was found in those receiving pegylated interferon-α-2b (see table II). At the end of the first week, a similar response to treatment was observed in the two arms. After 4 weeks of treatment, the $\log_{10}$ reduction and the rate of EVR did not significantly differ between the two groups.

Table V. Adverse effects observed during the treatment in the two study groups

Adverse effect	Pegylated interferon-α-2a (no. of pts)	Pegylated interferon-α-2b (no. of pts)
Influenza-like syndrome	24	21
Anaemia (Hb <10 g/dL)	7	6
Thrombocytopenia (<75 000 mm^3)	11	8
Leukopenia (<3000 mm^3)	7	5
Neutropenia (<750 mm^3)	0	0
Severe psychiatric disorders	3	3
Severe headache	1	1
Marked weight loss	3	2

Hb = haemoglobin; **pts** = patients.

The EVR pattern and the rate of HCV RNA clearance (22.2% vs 24.1%; p-value not significant) was similar in patients treated with pegylated interferon-α-2a compared with α-2b at week 12.

When comparing genotypes, no significant differences in EVR and SVR between the two pegylated interferons were observed in patients with genotypes 2 and 3. However, in patients with genotype 1, a lower percentage of slow partial response plus rapid virological response was reported in patients who received pegylated interferon-α-2a compared with those who received pegylated interferon-α-2b (10.8% vs 17.5%; p-value not significant). As for SVR, in patients with genotype 1, a similar response was reported with both pegylated interferons-α-2a and -α-2b (10.8% vs 10.0%), whereas, differences were found in baseline viral load with higher values in pegylated interferon-α-2a recipients compared with pegylated interferon-α-2b recipients.

When analysing genotype 4, it was observed that pegylated interferon-α-2a recipients presented a higher percentage of non-response in EVR compared with pegylated interferon-α-2b recipients (44.5% vs 25.0%), even though, when considering both non-responder patients and flat-partial responders, there was not a significant difference between the two groups (55.6% vs 50.0%) for this genotype. However, similar responses were observed in slow partial and rapid virological responders between the two groups and therefore in SVR.

When analysing the factors related to SVR, we observed that bodyweight, although referring to a small number of patients, could represent an important predictive factor. In previous studies of treatment-naive patients by Manns et al.,[6] Fried et al.[5] and Hadziyannins et al.,[29] different bodyweights (>75 or <75 kg) were associated with different SVRs. In our study, the patients treated with pegylated interferon-α-2a had a higher response percentage when the bodyweight was <75 kg, while in patients who weighed >75 kg, a more effective response was obtained with pegylated interferon-α-2b. This analysis seems to show that the standard dose of pegylated interferon-α-2a (180 μg/week) supplies a larger quantity of drug to patients with smaller bodyweight and a smaller quantity, especially to organs, such as the liver, to those with a larger bodyweight. A lower concentration of pegylated interferon-α-2a in the liver in patients with a larger bodyweight is likely to reflect the restricted biodistribution of this drug, such that it is predominantly found in the bloodstream and extracellular fluid at steady state. Although it has a longer half-life than pegylated interferon-α-2b, its volume of distribution is smaller.[16-19] In contrast, pegylated interferon-α-2b, with a dosage related to bodyweight, was able to assure that all patients received the appropriate quantity of drug. Therefore, these results, although obtained in a small cohort of subjects and so not statistically significant, seem to indicate that bodyweight is an important factor for SVR with α-2a treatment, while it would not be significant with α-2b therapy, suggesting that in patients with a higher bodyweight, administration of weight-related doses of pegylated interferon should be recommended.[30]

In conclusion, there are two pegylated interferons with different structural characteristics, but the pharmacodynamic effects reported in this pilot study regarding non-responder patients, demonstrated similar EVR and SVR for both drugs.

Obvious differences between pegylated interferon-α-2a and -α-2b exist, but currently, as a result of the small number of patients enrolled, we cannot state if these differences would have an impact on treatment outcome. We believe that these results should encourage multicentre trials, in particular in non-responders, who currently make up the majority of the population referred to our centres.

Acknowledgements

No sources of funding were used to assist in the preparation of this manuscript. The authors have no conflicts of interest that are directly relevant to the content of this article. The authors would like to thank all the participants in the study reported in this article.

References

1. Poynard T, Marcellin P, Lee SS, et al. Randomised trial of interferon alfa2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alfa2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet 1998; 352: 1426-32
2. Mchutchinson JG, Gordon SC, Schiff ER, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. N Engl J Med 1998; 339: 1485-92
3. Lam NP, Neumann AU, Gretch DR, et al. Dose-dependent acute clearance of hepatitis C genotype 1 virus with interferon alfa. Hepatology 1997; 26: 226-31
4. Davis GL, Esteban-Mur R, Risutgi V, et al. Recombinant interferon alfa-2b alone or in combination with ribavirin for retreatment on interferon relapse in chronic hepatitis C. N Engl J Med 1998; 339: 1493-9
5. Fried MW, Shiffman ML, Reddy RK, et al. Pegylated (40Kda) interferon alfa-2a (PEGASYS) in combination with ribavirin: efficacy and safety results from a phase III, randomised, actively-controlled, multicenter study [abstract]. Gastroenterology 2001; 120: A55
6. Manns P, Mchutchinson JG, Gordon SC, et al. Pegylated interferon alfa-2b in combination with ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: results of a randomised trial. Lancet 2001; 358: 958-65
7. Zeuzem S, Feinman VS, Rasenak J, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med 2000; 343: 1666-72
8. Bruno R, Sacchi P, Maiocchi L, et al. Host factors affecting the outcome of treatment of hepatitis C. Rev Gastroenterol Disord 2004; 4 Suppl. 1: 3-7
9. Zeuzem S, Schmidt JM, Lee J-H, et al. Effect of interferon alfa on the dynamics of hepatitis C virus turnover in vivo. Hepatology 1996; 23, 2: 366-71
10. Gavier B, Martinez-Gonzalez M-A, Riezu-Boj J-I, et al. Viremia after one month of interferon therapy predicts treatment outcome in patients with chronic hepatitis C. Gastroenterology 1997; 113: 1647-53
11. Neumann AU, Lam NP, Dahari H, et al. Hepatitis C viral in vivo and the antiviral efficacy of interferon alfa therapy. Science 1998; 282: 103-7
12. Brouwer JT, Hansen BE, Niesters HGM, et al. Early prediction of response in interferon monotherapy and in interferon-ribavirin combination therapy for chronic hepatitis C: HCV RNA at 4 weeks versus ALT. J Hepatol 1999; 30: 192-8

13. Zeuzem S, Herrmann E, Lee J-J, et al. Viral kinetic in patients with chronic hepatitis C treated with standard or peginterferon alfa-2a. Gastroenterology 2001; 120: 1438-47
14. Davis GL, Wong JB, McHutchinson JG, et al. Early virological response to treatment with peginterferon alfa-2bplus ribavirin in patients with chronic hepatitis C. Hepatology 2003; 38, 3: 645-52
15. Davis GL. Monitoring of viral levels during therapy of hepatitis C. Hepatology 2002; 36, 5 Suppl. 1: 145-51
16. Harris JM, Martin NE, Modi M. Pegylation: a novel process for modifyng pharmacokinetics. Clin Pharmacokinet 2001; 40 (7): 539-51
17. Kozlowski A, Charles SA, Harris JM. Development of pegylated interferons for the treatment of chronic hepatitis C. BioDrugs 2001; 15 (7): 419-29
18. Bailon P, Palleroni A, Schaffer CA, et al. Rational design of a potent, long-lasting form of interferon: a 40 Kda branched polyethylene glicol-conjugated interferon alfa-2a for the treatment of hepatitis C. Bioconjug Chem 2001; 12 (2): 195-202
19. Glue P, Fang JM, Rouzier-Panis R, et al. Pegylated interferon-alfa 2b: pharmacokinetics, pharmacodynamics, safety, and preliminary efficacy data. Hepatitis C Intervention Therapy Group. Clin Pharmacol Ther 2000; 68 (5): 556-67
20. Bruno R, Sacchi P, Ciappina V, et al. Viral dynamics and pharmacokinetics of peginterferon alfa-2a and peginterferon alfa-2b in naive patients with chronic hepatitis C: a randomised, controlled study. Antivir Ther: 2004; 9 (4): 491-7
21. Silva M, Poo J, Wagner F, et al. A randomised trial to compare the pharmacokinetic, pharmacodynamic, and antiviral effects of peginterferon alfa-2b and peginterferon alfa-2a in patients with chronic hepatitis C (COMPARE). J Hepatol 2006; 45: 204-13
22. Shiffman ML. Retreatment of patients with chronic hepatitis C. Hepatology 2002; 36: S128-34
23. Buti M, Sanchez-Avila F, Lurie Y, et al. Viral kinetics in genotype 1 chronic hepatitis C patients during therapy with 2 different doses of peginterferon alfa-2b plus ribavirin. Hepatology 2002; 35 (4): 930-6
24. Jessner W, Stauber R, Hackl F, et al. Early viral kinetics on treatment with pegylated interferon alfa-2a in chronic hepatitis C virus genotype 1 infection. J Viral Hepatitis 2003; 10: 37-42
25. Bekkering FC, Brouwer JT, Leroux-Roels G, et al. Ultrarapid hepatitis c virus clearance by daily high-dose interferon in non-responders to standard therapy. J Hepatol 1998; 28: 960-4
26. Cotler SJ, Layden JE, Neumann AU, et al. First phase hepatitis C viral kinetics in previous non-responders patients. J Viral Hepatitis 2003; 10: 43-9
27. Ferenci P. Predicting the therapeutic response in patients with chronic hepatitis C: the role of viral kinetics studies. J Antmic Chemot 2004; 53: 15-8
28. Layden JE, Layden TJ, Levi-Drummer R, et al. Early viral kinetics of HCV-predicting treatment response after only 24 hours (Abs). Digestive Disease Week; 2001 May 20-23; Atlanta (GA); DDW Program Book, AGA 2001, 30
29. Hadziyannis SJ, Cheinquer H, Morgan T, et al. Peginterferon alfa-2a (40 Kda) (PEGASYS) in combination with ribavirin (RBV): efficacy and safety results from a phase III, randomised, double-blind multicentre study examining effect of duration of treatment and RBV dose [abstract]. J Hepatol 2002; 36 Suppl. 1: 3
30. Alberti A, Benvegnù L. Management of hepatitis C. J Hepatol 2003; 38: S104-18

Correspondence: *Gaetano Scotto*, MD, Clinic of Infectious Diseases, University of Foggia, Luigi Pinto Street, 1α, 71100 Foggia, Italy.
E-mail: gaescot@tin.it