Phase 1b Study of Pegylated Interferon Lambda 1 With or Without Ribavirin in Patients with Chronic Genotype 1 Hepatitis C Virus Infection

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Interferon lambda 1 (IFN-λ1) is a type III IFN that produces intracellular responses similar to those of IFN-α but in fewer cell types because of differences in the receptor distribution pattern, and this could potentially result in an improved safety profile. This was an open-label three-part study of patients with chronic hepatitis C virus (HCV) genotype 1 infection. Part 1 evaluated single-agent pegylated interferon lambda (PEG-IFN-λ) at 1.5 or 3.0 μg/kg administered every 2 weeks or weekly for 4 weeks in patients who had relapsed after previous IFN-α-based treatment. Part 2 evaluated weekly doses of PEG-IFN-λ ranging from 0.5 to 2.25 μg/kg in combination with ribavirin (RBV) for 4 weeks in treatment-relapse patients. Part 3 evaluated weekly PEG-IFN-λ at 1.5 μg/kg in combination with RBV for 4 weeks in treatment-naive patients. Fifty-six patients were enrolled: 24 patients in part 1, 25 patients in part 2, and 7 patients in part 3. Antiviral activity was observed at all PEG-IFN-λ dose levels (from 0.5 to 3.0 μg/kg). Two of seven treatment-naive patients (29%) achieved rapid virological response. Treatment was well tolerated with minimal flu-like symptoms and no significant hematologic changes other than RBV-associated decreases in hemoglobin. The most common adverse events were fatigue (29%), nausea (12%), and myalgia (11%). Six patients experienced increases in aminotransferases that met protocol-defined criteria for dose-limiting toxicity (DLT) or temporarily holding therapy with PEG-IFN-λ. Most DLT occurred in patients with high PEG-IFN-λ exposure. *Conclusion:* Weekly PEG-IFN-λ with or without daily RBV for 4 weeks is well tolerated with minimal adverse events and hematologic effects and is associated with clear antiviral activity across a broad range of doses in patients with chronic HCV. (HEPATOLOGY 2010;00:000-000)

The World Health Organization estimates that 180 million people worldwide (3% of the world population) are infected with hepatitis C virus (HCV), and 130 million of these are chronic HCV carriers.[1] Chronic HCV infection is responsible for 50% to 76% of all liver cancer cases, two-thirds of

all liver transplants in the developed world, and considerable morbidity and mortality. Identifying effective treatments for chronic HCV infection is therefore a global health priority.[1]

Consensus guidelines for the treatment of hepatitis C recommend a regimen of pegylated interferon alfa (PEG-IFN-α) and ribavirin (RBV).[2] However, this treatment regimen results in sustained virological response (SVR) rates of only 40% in patients with genotype 1 HCV, and associated adverse events include flu-like symptoms, fatigue, depression, anxiety, and bone marrow suppression, which results in anemia, neutropenia, and thrombocytopenia.[2-6] PEG-IFN-α is contraindicated in patients with major uncontrolled depressive illness and should be used with caution in patients with any psychiatric illness.[2,5-7] Other contraindications for the use of interferon alpha (IFN-α)–based regimens include hepatic decompensation, autoimmune disease, and severe concurrent medical disease such as chronic obstructive pulmonary disease, congestive heart failure, or significant coronary artery disease.[2,6,7] In addition to the negative impact on quality of life, adverse events and laboratory abnormalities often lead to dose reductions or discontinuation of IFN-α therapy, which further compromises efficacy.[2,3,8,9]

IFN-λ1, also known as interleukin-29 (IL-29), is a type III IFN with functional similarities to type I IFNs, which include IFN-α and IFN-β. Like IFN-α and IFN-β, IFN-λ1 is induced in response to viral infections such as hepatitis C and has demonstrated antiviral activity *in vitro*, including inhibition of HCV RNA replication in the replicon model.[10] IFN-λ1 interacts with the structurally unique IFN-λ1 receptor complex to stimulate an intracellular response through phosphorylation of the Janus kinase/signal transducer and activator of transcription pathway (similar to the mechanism of action of IFN-α) and leads to the upregulation of IFN-stimulated genes and an antiviral effect.[11,12] Unlike the widely distributed IFN-α receptor, expression of the IFN-λ1 receptor is more restricted. Although all cell types in the liver express the IFN-α receptor, the IFN-λ1 receptor is found only in hepatocytes. Similarly, although all peripheral blood leukocytes, including B, T, and natural killer cells, neutrophils, and monocytes, express the IFN-α receptor, messenger RNA of the IFN-λ1 receptor is not expressed in hematopoietic cells with the exception of B lymphocytes.[10,13] The limited distribution of the IFN-λ1 receptor suggests the potential for reduced adverse events with IFN-λ1–based therapy in comparison with IFN-α–based therapy along with preservation of the antiviral effect in HCV.

PEG-IFN-λ, a conjugate of a recombinant form of human IFN-λ1 and a 20-kDa linear polyethylene glycol chain, is currently under development for the treatment of chronic HCV infection. A phase 1a, placebo-controlled, dose escalation study of single subcutaneous doses of PEG-IFN-λ in healthy subjects was recently completed.[14] PEG-IFN-λ was well tolerated at pharmacologically active doses without the toxicities typically associated with PEG-IFN-α. The estimated half-life was 50 to 80 hours, and the time to the maximum concentration was 8 to 24 hours. PEG-IFN-λ, starting at the 1.5 μg/kg dose, demonstrated dose-dependent biological activity with the induction of increases in serum β2-microglobulin (β2M). Here we describe the results of a three-part study assessing the safety and antiviral activity of PEG-IFN-λ with or without RBV over 4 weeks in patients infected with genotype 1 chronic HCV.

Patients and Methods

Study Design. This was a three-part dose and schedule escalation study of PEG-IFN-λ administered subcutaneously as a single agent or in combination with RBV in patients chronically infected with HCV genotype 1 who had relapsed after IFN-α–based treatment (parts 1 and 2) or who were naive to treatment (part 3). Part 1 of the study evaluated escalating doses of PEG-IFN-λ monotherapy administered either every 2 weeks (Q2W) or weekly (QW) for a total of 4 weeks. Parts 2 and 3 of this study evaluated a range of doses of PEG-IFN-λ administered QW in combination with RBV twice daily for 4 weeks. All patients were followed for at least 4 weeks after the completion of treatment.

Figure 1 summarizes the treatment schema for the three parts of the study. PEG-IFN-λ doses ranging from 0.5 to 3.0 μg/kg were evaluated. PEG-IFN-λ was supplied at a concentration of 10 mg/mL, and a two-step dilution was required to achieve the final dose for subcutaneous administration. No dose modifications were allowed. In parts 2 and 3, RBV (Copegus, Roche Laboratories, Inc., Nutley, NJ) was administered orally twice daily to achieve a total dose of 1000 mg for patients < 75 kg or 1200 mg for patients $\geq$ 75 kg. The primary endpoints of the study were safety and tolerability. Secondary endpoints included HCV RNA reduction and pharmacokinetic analysis of PEG-IFN-λ serum concentrations.

Part 1: Single-agent therapy, IFN-α relapse patients

| 1.5 μg/kg Q2W n=6 | 3.0 μg/kg Q2W n=6 | 1.5 μg/kg QW n=6 | 3.0 μg/kg QW n=6 |

Part 2: Combination therapy with ribavirin (RBV), IFN-α relapse patients

| 0.5 μg/kg QW + RBV n=6 | 0.75 μg/kg QW + RBV n=6 | 1.5 μg/kg QW + RBV n=7 | 2.25 μg/kg QW + RBV n=6 |

Part 3: Combination therapy with ribavirin (RBV), treatment-naïve patients
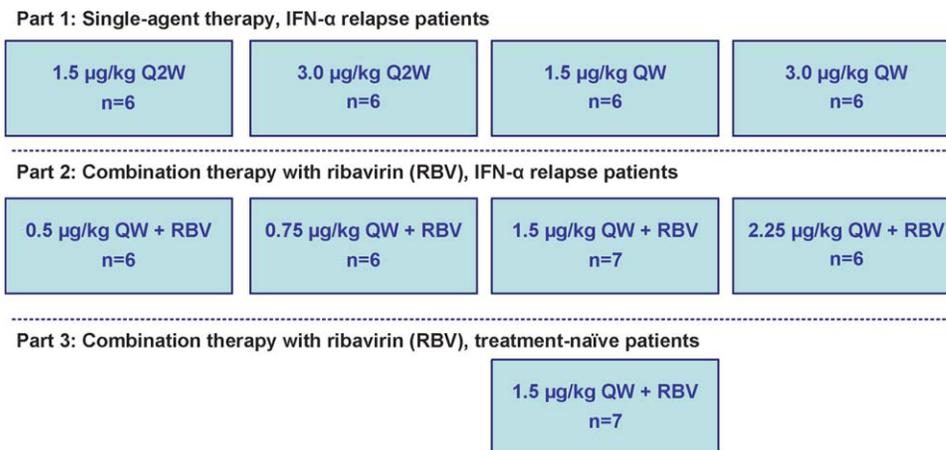
| 1.5 μg/kg QW + RBV n=7 |

Fig. 1. Study schema.

Each cohort consisted of at least six evaluable patients. To be considered evaluable, a patient had to have completed all study visits through day 29 (Q2W cohorts) or day 36 (QW cohorts) unless the reason for not doing so was PEG-IFN-λ–related toxicity. A dose level or schedule was considered not tolerated if two or more patients experienced dose-limiting toxicity (DLT) or if two or more patients were unable to receive all planned doses because of treatment-related toxicity. Data from each cohort were reviewed by a safety monitoring committee.

Patients. Parts 1 and 2 enrolled patients with chronic HCV infection who had relapsed after at least 12 weeks of prior treatment for HCV with either PEG-IFN-α or another IFN-α in combination with RBV. Part 3 enrolled treatment-naive patients. Patients had HCV genotype 1 infection and serum HCV RNA levels $\geq$ 100,000 IU/mL at enrollment. Inclusion criteria included alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels $\leq$ 2.5 times the upper limit of normal with no evidence of decompensated liver disease or hepatocellular carcinoma and documented liver biopsy within 2 years of study enrollment with an Ishak score $\leq$ 4.[15] Patients were excluded if they had significant cardiac disease or a medical condition requiring immunosuppressive therapy. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was consistent with good clinical practice guidelines and requirements of the regulatory authorities at each institution. All patients provided written, informed consent.

Safety Assessments. All patients who received at least one dose of PEG-IFN-λ were included in the safety analyses. The evaluation of safety included adverse event monitoring, physical examination, clinical laboratories (hematology and serum chemistry),

electrocardiogram, and echocardiogram. The National Cancer Institute's Common Terminology Criteria for Adverse Events (version 3.0) was used to evaluate adverse event severity and laboratory toxicities.

DLT included any clinical adverse event $\geq$ grade 3 in severity that was at least possibly related to PEG-IFN-λ treatment, with the exceptions of transient (<72-hour) grade 3 fatigue, fever, or rigor. In addition, a >5-fold increase in ALT or AST levels from the baseline ($\geq$grade 2) or a >2-fold increase in ALT or AST levels from the baseline ($\geq$grade 2) with a grade 2 elevation in bilirubin was considered DLT. If the ALT or AST level increased to more than 3 times the baseline ($\geq$grade 2) or more than 7 times the upper limit of normal, but the bilirubin level or international normalized ratio did not increase to grade 2, PEG-IFN-λ was to be held until the ALT or AST level returned to less than 2 times the baseline value.

Pharmacokinetics. Pharmacokinetic samples were collected at selected time points throughout the study. Samples were analyzed with a fully validated method developed at ZymoGenetics with mesoscale discovery electrochemiluminescent technology to quantify the PEG-IFN-λ serum concentration. The lower limit of quantification of the assay was 0.125 ng/mL in human serum. The PEG-IFN-λ serum concentration versus time profile for each patient was evaluated by noncompartmental methods with the software WinNonlin 5.2.1 (Pharsight Corp., Cary, NC). The maximum serum concentration (C_{max}) and the area under the curve (AUC_{0-t}) were estimated for each patient.

Immunogenicity. Serum samples for evaluating antibody responses directed against PEG-IFN-λ were collected and analyzed with a fully validated method developed at ZymoGenetics with mesoscale discovery electrochemiluminescent technology to detect antibodies to PEG-IFN-λ1. Analysis was performed by a

Table 1. Summary of the Patient Demographics and Baseline Disease Characteristics

	Part 1: IFN-α Relapse, Single-Agent PEG-IFN-λ (n = 24)	Part 2: IFN-α Relapse, PEG-IFN-λ and RBV (n = 25)*	Part 3: Treatment-Naive, PEG-IFN-λ and RBV (n = 7)	Total (n = 56)
Age (years), mean (range)	52.9 (43-66)	51.6 (24-59)	43.6 (35-49)	51.1 (24-66)
Gender, n (%)				
Female	10 (42)	6 (24)	2 (29)	18 (32)
Male	14 (58)	19 (76)	5 (71)	38 (68)
Race, n (%)				
African American	3 (13)	4 (16)	2 (29)	9 (16)
Hispanic	9 (38)	2 (8)	2 (29)	13 (23)
White	12 (50)	19 (76)	3 (43)	34 (61)
BMI (kg/m^2), mean (range)	29.74 (23.8-48.7)	30.44 (21.1-39.1)	29.54 (26.5-35.9)	30.03 (21.1-48.7)
HCV RNA at baseline, log$_{10}$ mean (range)	6.55 (4.21-7.56)	6.36 (5.37-7.52)	6.26 (5.80-6.85)	6.43 (4.21-7.56)

Abbreviation: BMI, body mass index.

*One patient discontinued treatment because of an unrelated adverse reaction to the drug meperidine and was excluded from the efficacy analyses.

tiered approach designed to confirm reactivity, quantify the antibody titer, and demonstrate specificity. Samples that were confirmed to be reactive and demonstrated specificity to the drug product were assayed for neutralizing activity in a cell-based assay. The lower limit of assay detection was 0.1 μg/mL. The cell-based neutralizing bioassay was qualitative in nature.

Pharmacodynamics. Samples were collected for pharmacodynamic studies at selected time points during the study. β2M levels were assessed with a competitive binding assay (R&D Systems, Minneapolis, MN). The lower limit of assay quantification was 0.4 μg/mL.

Efficacy Assessments. HCV RNA levels were measured with the COBAS TaqMan HCV test (version 2.0, Roche Molecular Diagnostics, Pleasanton, CA) with a lower limit of detection of 25 IU/mL. A central laboratory with expertise in the serial measurement of HCV RNA levels was used (Covance, Indianapolis, IN).

Data Analysis Methods. No formal hypothesis was proposed; however, a cohort size of six patients would allow for a greater than 80% probability of detecting an event with a true population incidence of 25% and allow an evaluation of antiviral activity. The presented data represent an intent-to-treat population. All statistical analyses were performed with SAS (version 9.1.3 or higher, SAS Institute, Inc., Cary, NC) or other commercially available validated software.

Results

Patient Demographics and Baseline Characteristics. Fifty-six patients were enrolled from 10 sites in the United States: 24 with treatment-relapse disease in part 1, 25 with treatment-relapse disease in part 2, and 7 with treatment-naive disease in part 3 (Fig. 1).

All but one patient completed the study through day 59. One patient in the cohort receiving 1.5 μg/kg PEG-IFN-λ QW plus RBV discontinued dosing after receiving PEG-IFN-λ through day 8 because of an adverse drug reaction to meperidine that was deemed unrelated to PEG-IFN-λ. Data from this patient were excluded from the efficacy assessment.

Patient demographics are shown in Table 1. All treatment-relapse patients had received at least one previous course of therapy with a PEG-IFN-α plus RBV except for two patients, one of whom had previously received treatment with albinterferon alfa-2b plus RBV and one of whom had previously received interferon alfacon-1.

Safety and Tolerability. PEG-IFN-λ was well tolerated by most patients. Four of 56 patients (7%) had one or more doses withheld because of treatment-related toxicity. Most adverse events were mild or moderate in severity. Table 2 summarizes adverse events occurring in at least three patients in all cohorts combined. The most common adverse events were fatigue (29%), nausea (12%), myalgia (11%), and headache (9%). Among treatment-relapse patients, fatigue, nausea, myalgia, headache, diarrhea, irritability, pruritis, and insomnia were more commonly observed in patients treated with PEG-IFN-λ in combination with RBV versus those receiving single-agent therapy. PEG-IFN-λ plus RBV therapy was very well tolerated by treatment-naive patients, with fatigue, headache, and chills each reported by one of seven patients (14%) and with no patients reporting nausea, myalgia, diarrhea, irritability, pruritis, anorexia, influenza-like illness, or insomnia. The incidence of adverse events did not appear to be dose-related; adverse events were reported in 33.3%, 50%, 50%, and 50% of patients in part 1 cohorts receiving 1.5 μg/kg PEG-IFN-λ Q2W, 3.0 μg/kg PEG-IFN-λ Q2W, 1.5 μg/kg PEG-

Table 2. Summary of Adverse Events (for Three or More Patients in All Cohorts Combined) by the MedRA Preferred Terms

MedRA Preferred Term*	Part 1: IFN-α Relapse, Single-Agent PEG-IFN-λ (n = 24)	Part 2: IFN-α Relapse, PEG-IFN-λ and RBV (n = 25)	Part 3: Treatment-Naive, PEG-IFN-λ and RBV (n = 7)	Total (n = 56)
Fatigue, n (%)	3 (12.5)	12 (48.0)	1 (14.3)	16 (28.6)
Nausea, n (%)	2 (8.3)	5 (20.0)	0 (0.0)	7 (12.5)
Myalgia, n (%)	2 (8.3)	4 (16.0)	0 (0.0)	6 (10.7)
Headache, n (%)	0 (0.0)	4 (16.0)	1 (14.3)	5 (8.9)
Diarrhea, n (%)	1 (4.2)	3 (12.0)	0 (0.0)	4 (7.1)
Irritability, n (%)	1 (4.2)	3 (12.0)	0 (0.0)	4 (7.1)
Pruritis, n (%)	0 (0.0)	4 (16.0)	0 (0.0)	4 (7.1)
Anorexia, n (%)	2 (8.3)	1 (4.0)	0 (0.0)	3 (5.4)
Chills, n (%)	0 (0.0)	2 (8.0)	1 (14.3)	3 (5.4)
Influenza-like illness, n (%)	1 (4.2)	2 (8.0)	0 (0.0)	3 (5.4)
Insomnia, n (%)	0 (0.0)	3 (12.0)	0 (0.0)	3 (5.4)

Abbreviation: MedRA, Medical Dictionary for Regulatory Affairs.

*Multiple occurrences of an event for a patient were counted only once.

IFN-λ QW, and 3.0 μg/kg PEG-IFN-λ QW, respectively. Similarly, in part 2 (PEG-IFN-λ plus RBV), adverse events were reported in 83.3%, 83.3%, 85.7%, and 83.3% of patients receiving 0.5 μg/kg PEG-IFN-λ Q2W, 0.75 μg/kg PEG-IFN-λ Q2W, 1.5 μg/kg PEG-IFN-λ QW, and 2.25 μg/kg PEG-IFN-λ QW, respectively. Adverse events were reported in 42.9% of treatment-naive patients receiving 1.5 μg/kg PEG-IFN-λ QW plus RBV.

Two patients experienced other clinically important events considered related to PEG-IFN-λ. One patient treated with 3.0 μg/kg PEG-IFN-λ QW as a single agent experienced grade 3 idiopathic thrombocytopenic purpura, which occurred 2 weeks after the final dose; this event was considered DLT. The patient responded rapidly to therapy with oral prednisone. Another patient, treated with 1.5 μg/kg PEG-IFN-λ QW plus RBV in part 2, experienced elevated ALT, AST, and bilirubin levels, which met the protocol-defined criteria for withdrawing therapy. However, this patient received an additional dose of PEG-IFN-λ in violation of the protocol and subsequently experienced further elevation of ALT, AST, and bilirubin levels, which resulted in suspected medication-associated hepatotoxicity, pruritis, and elevated lipase and amylase levels (without associated clinical symptoms of pancreatitis).

Clinical Laboratory Evaluations. Five patients experienced aminotransferase elevations that met the protocol-defined criteria for DLT, and an additional patient met the criteria for temporarily holding of a dose of PEG-IFN-λ (Table 3). Of these patients, four were in cohorts receiving the highest PEG-IFN-λ dose evaluated [3.0 μg/kg QW (n = 3) or Q2W (n = 1)], and two were in cohorts receiving 1.5 μg/kg PEG-IFN-λ QW with RBV (n = 1) or without RBV (n = 1). In three patients, the ALT and AST elevations were accompanied by a bilirubin elevation. All elevations in ALT, AST, and bilirubin were reversible, generally at or before the end of the study visit (day 59).

Table 3. ALT, AST, and Bilirubin Levels by Visit Day for Patients with DLT or Patients with the Dose Withheld Because of Increases in ALT or AST

Cohort/Patient	ALT (U/L)/AST (U/L)/Bilirubin (μmol/L)					
	Day 1	Day 8	Day 15	Day 22	Day 29	Day 59
3.0 μg/kg Q2W/502-0019*	46/40/10.3	54/55/6.8	43/39/8.5	126/204/8.5	65/45/6.8	49/42/8.5
1.5 μg/kg QW/506-0032*	39/32/17.1	105/89/15.4	172/134/15.4‖	179/182/27.4‖	264/167/46.2	53/37/12.0
1.5 μg/kg QW and RBV/506-0035*,†	96/102/27.4	88/103/27.4	125/144/63.6	528/847/198.4‖	370/514/357.5	116/148/65.0
3.0 μg/kg QW/502-0061*,‡	64/46/8.5	115/104/47.9	153/114/34.2	91/70/17.1	94/69/13.7	70/54/10.3
3.0 μg/kg QW/502-0065*,§	29/36/8.5	56/41/8.5	127/165/6.8	173/180/8.5	63/48/10.3	20/24/8.5
3.0 μg/kg QW/502-0066§	107/67/6.8	152/109/6.8	154/121/6.8	241/229/6.8	65/37/8.5	118/79/10.3

*The patient met the criteria for DLT according to hepatic laboratory values.

†The maximum values of ALT and AST on day 26 were 667 and 1328 U/L, respectively (bilirubin level = 348.9 μmol/L).

‡The patient did not receive the day 15 or day 22 dose. The patient also experienced a grade 2 elevation of bilirubin and a grade 3 elevation of lipase on days 8 and 15.
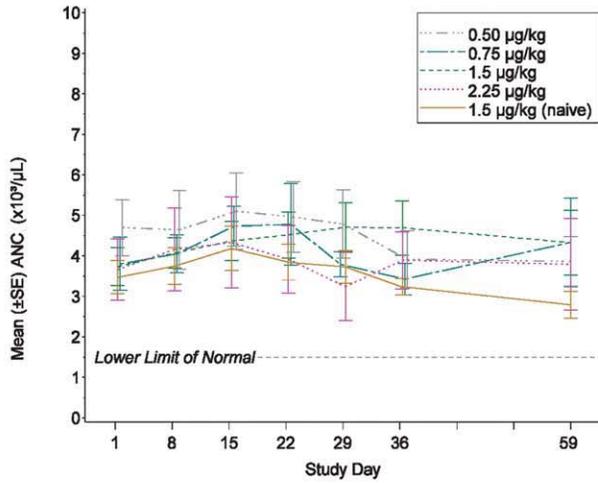
§The patient did not receive the day 22 dose.

‖The study drug was administered even though the protocol-defined criteria for withholding the dose were met.

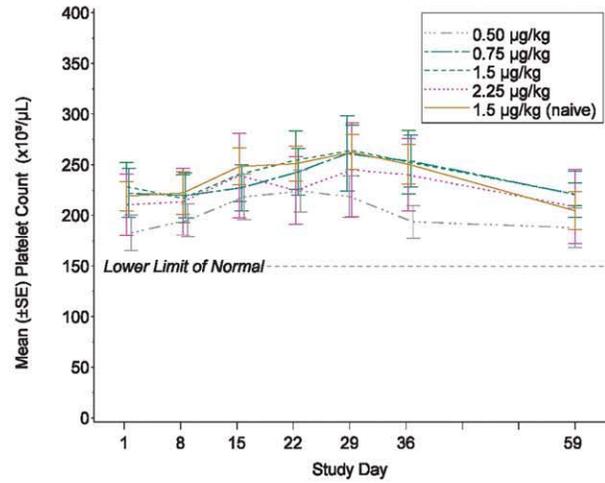¶1 μmol/L bilirubin = 0.0584 mg/dL bilirubin.

A1

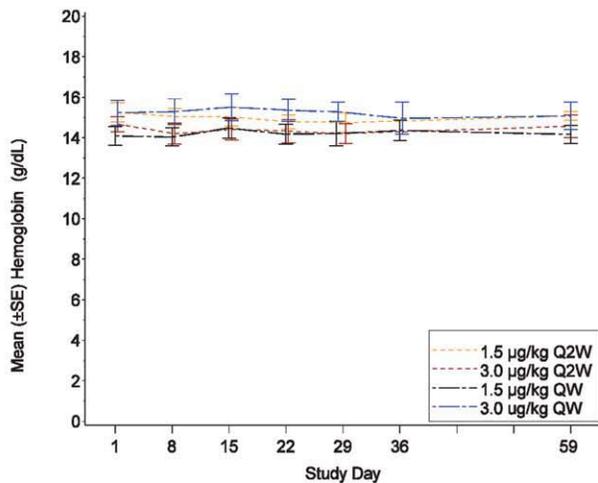**Mean absolute neutrophil count over time · weekly dosing + ribavirin cohorts**

A2

Mean platelet count over time – weekly dosing cohorts + ribavirin cohorts

B1

Mean hemoglobin over time – single–agent cohorts

B2

Mean hemoglobin over time - weekly dosing + ribavirin cohorts
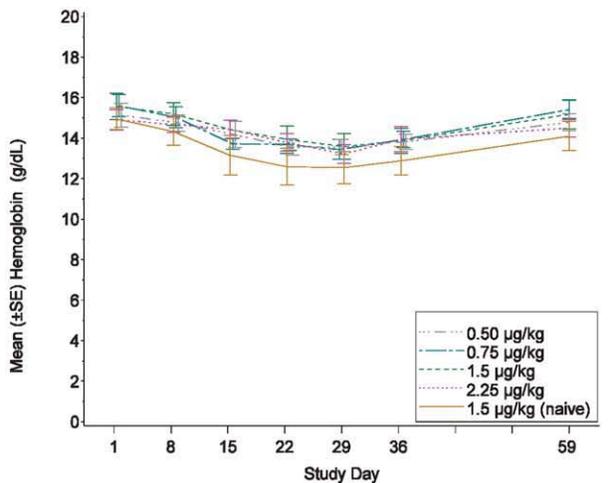

Fig. 2. Select hematologic laboratory parameters over time. Abbreviations: ANC, absolute neutrophil count; SE, standard error.

Grade 3 or 4 elevations in lipase and/or amylase not associated with abdominal pain or nausea were observed in four patients during the study; all were resolved after the withholding or discontinuation of PEG-IFN-λ. One patient had the day 15 dose held and was subsequently successfully treated on day 22, with no loss of antiviral effect observed that was related to the interruption. No other clinically important changes were noted in serum chemistry values.

Mean absolute neutrophil counts and platelet counts over time in cohorts receiving PEG-IFN-λ plus RBV are displayed in Fig. 2(A1,A2), respectively. There were no clinically significant changes in absolute neutrophil counts. Three patients with baseline absolute neutrophil counts in the low normal range of 2.04 to

2.30×10^9/L had decreases to 1.62 to 1.80×10^9/L; these were all isolated values that required no change in the studied therapy. One patient (described previously) experienced a grade 3 decrease in platelets during the posttreatment follow-up period, and this was concurrent with a diagnosis of idiopathic thrombocytopenic purpura; platelet decreases were not observed in other patients. Figure 2(B1,B2) displays the mean hemoglobin values in the cohorts that received PEG-IFN-λ as a single agent and PEG-IFN-λ plus RBV, respectively. No significant changes were observed in patients treated with single-agent PEG-IFN-λ, whereas decreases in hemoglobin consistent with the known effects of RBV were observed in patients treated with combination therapy.

Table 4. Summary of Virological Responses

	Part 1: Treatment Relapse, Single-Agent PEG-IFN-λ				Part 2: Treatment Relapse, PEG-IFN-λ and RBV				Part 3: Treatment-Naive, PEG-IFN-λ and RBV
	1.5 μg/kg Q2W (n = 6)	3.0 μg/kg Q2W (n = 6)	1.5 μg/kg QW (n = 6)	3.0 μg/kg QW (n = 6)	0.5 μg/kg QW (n = 6)	0.75 μg/kg QW (n = 6)	1.5 μg/kg QW (n = 6)†	2.25 μg/kg QW (n = 6)	1.5 μg/kg QW (n = 7)
Serum HCV RNA (log$_{10}$), mean (range)									
Baseline	7.34 (7.08-7.56)	6.47 (6.11-7.17)	6.58 (5.93-7.38)	5.79 (4.21-6.57)	6.17 (5.52-6.60)	6.38 (5.37-7.20)	6.70 (5.89-7.52)	6.18 (5.66-6.92)	6.26 (5.80-6.85)
Maximum decrease from the baseline	2.15 (0.59-5.18)	1.89 (0.98-3.01)	3.60 (2.05-4.95)	3.42 (2.49-4.60)	1.83 (0.49-3.57)	3.00 (0.70-4.66)	3.25 (0.09-5.56)	3.85 (3.20-5.14)	3.27 (1.23-5.45)
Virological response, n (%)									
$\geq$2-log$_{10}$ decrease in HCV RNA	2 (33)	3 (50)	6 (100)	6 (100)	2 (33)	4 (67)	5 (83)	6 (100)	6 (86)
HCV RNA < 1000 IU/mL	1 (17)	0	4 (67)	4 (67)	1 (17)	3 (50)	2 (33)	6 (100)	3 (43)
HCV RNA < 25 IU/mL*	0	0	0	3 (50)	0	1 (17)	0	1 (17)	2 (29)

*The lower limit of detection for HCV RNA was <25 IU/mL.

†This excludes one patient who discontinued treatment because of an unrelated adverse drug reaction to meperidine.

Antiviral Activity. Virological responses are summarized in Table 4. Antiviral activity was observed at all PEG-IFN-λ dose levels. Among the 43 patients dosed QW and evaluable for efficacy, all but 4 achieved a >1-log$_{10}$ decline in HCV RNA during the study. The first day of treatment yielded a rapid decline in HCV RNA that continued in a biphasic manner from days 4 through 29; this was similar to the pattern observed with IFN-α.[16] Higher PEG-IFN-λ doses were associated with greater declines in HCV RNA when PEG-IFN-λ was used both as a single agent and in combination with RBV (Fig. 3). At PEG-IFN-λ doses $\geq$ 1.5 μg/kg QW with or without RBV, 23 of 24 treatment-relapse patients (96%) achieved a >2-log$_{10}$ decline in HCV RNA levels, and 4 of 24 (17%) achieved undetectable HCV RNA. Among treatment-naive patients, six of seven patients (86%) achieved a >2-log$_{10}$ decline in HCV RNA levels, and they included two (29%) who achieved undetectable HCV RNA. Among patients dosed QW with PEG-IFN-λ with or without RBV, a >2-log$_{10}$ decline in HCV was achieved by 21 of 28 white patients (75%), 8 of 8 African American patients (100%), and 6 of 7 Hispanic patients (86%).

Four treatment-relapse patients who received PEG-IFN-λ QW did not achieve a >1-log$_{10}$ reduction in HCV RNA. Three of these patients were in the lowest PEG-IFN-λ dose cohorts; two were treated with 0.5 μg/kg PEG-IFN-λ QW plus RBV, and one was treated with 0.75 μg/kg PEG-IFN-λ QW plus RBV. The remaining patient without a detectable antiviral response was treated with 1.5 μg/kg PEG-IFN-λ QW plus RBV; this patient had preexisting neutralizing antibodies against PEG-IFN-λ and very low or undetectable serum levels of the study drug throughout the study period.

Pharmacokinetics. The first-dose C_{max} and AUC$_{0-t}$ values are summarized in Table 5. Two patients were excluded from the pharmacokinetic analyses; one patient who received PEG-IFN-λ (1.5 μg/kg) was excluded because of premature discontinuation of the study, and one patient who received 0.5 μg/kg PEG-IFN-λ was excluded because of C_{max} and AUC$_{0-t}$ values grossly inconsistent with the nominal dose administered.

Overall, the exposure of PEG-IFN-λ appeared to be dose-dependent and ranged from a mean AUC$_{0-t}$ value of 11.6 h*ng/mL at the 0.5 μg/kg dose level to 148 h*ng/mL at the 3.0 mg/kg dose level. Normalization of AUC$_{0-t}$ by dose (μg/kg) appeared to remove the effect of dose on exposure, and this suggested that exposure may increase linearly with dose. A modest
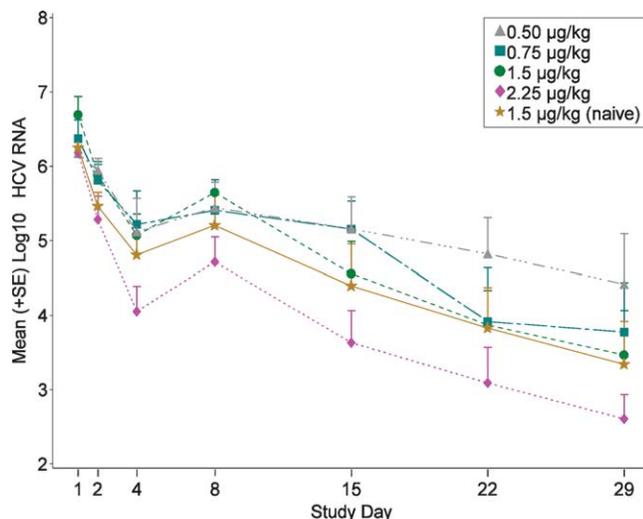
Fig. 3. Mean HCV RNA levels ($\log_{10}$ IU/mL) over time (QW plus RBV cohorts). HCV RNA levels were evaluated before PEG-IFN-λ dosing on days 1, 8, 15, and 22. Abbreviation: SE, standard error.

Fig. 4. Relationship between the first-dose PEG-IFN-λ AUC_{0-t} values and the changes in the HCV RNA levels ($\log_{10}$) among patients receiving PEG-IFN-λ QW. AUC_{0-t} values for some patients that were not estimated because of a lack of quantifiable data were imputed to be one-half of the lowest reported AUC_{0-t} value (6.78 h*ng/mL/2 = 3.39 h*ng/mL). Three patients were excluded: one who achieved undetectable HCV RNA levels but did not have a 1-week change in HCV RNA reported, one who discontinued the study prematurely because of an adverse reaction to the drug meperidine, and one with an AUC_{0-t} value inconsistent with the nominal dose administered. Abbreviation: RVR, rapid virological response.

accumulation from week 1 to week 4 was observed with average patient C_{max} and AUC_{0-t} accumulation index values of 1.12 and 1.34, respectively. No obvious effect of RBV on the exposure to PEG-IFN-λ was detected. To explore the effect of body weight on exposure, AUC_{0-t}/dose (μg) estimates were compared to the body weight. No trend was detected, and linear regression analysis indicated no apparent effect of weight on exposure.

Although there was no continuous relationship between exposure and change in ALT, very high exposure was observed in patients who demonstrated significant ALT elevations. Among patients dosed QW, six had a PEG-IFN-λ AUC_{0-t} value > 225 h ng/mL; four of these patients (67%) experienced DLT. Conversely, there appeared to be a continuous relationship between PEG-IFN-λ exposure and decreases in HCV RNA, with robust antiviral activity observed at exposures both above and below 225 h*ng/mL (Fig. 4).

Pharmacodynamics. A rapid rise in β2M levels was observed after the first dose of PEG-IFN-λ; levels peaked on day 4 and decreased toward the baseline
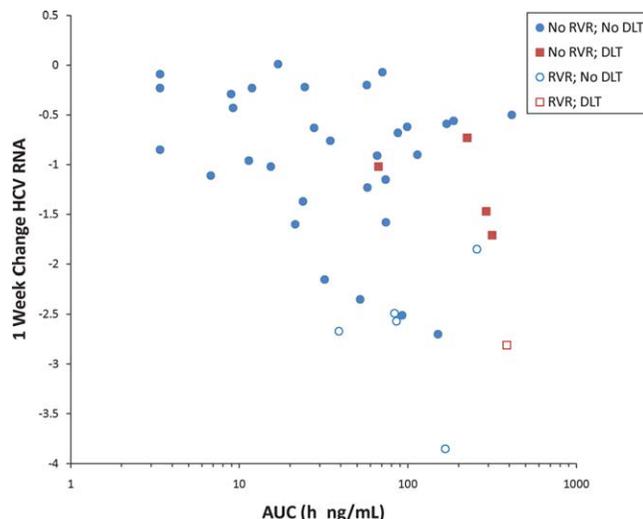
values by day 8. Increases in β2M were observed at all dose levels, and this confirmed pharmacological activity. A potential relationship between β2M induction and decreases in HCV RNA was observed. In general, patients who had the greatest HCV RNA decline on day 8 had the strongest induction of β2M. In addition, minimal or no induction of β2M was observed in patients with only minimal changes in HCV RNA on day 8, and this suggested that the lack of a virological response in these patients was likely related to an absence of pharmacological activity.

Immunogenicity. Low-titer antibodies specific to PEG-IFN-λ developed in 3 of 56 patients (5.4%) during the study (all in the treatment-relapse group). Neutralizing activity was observed on day 59 in one of

Table 5. First-Dose Pharmacokinetic Parameters

	0.5 μg/kg (n = 5)*	0.75 μg/kg (n = 6)	1.5 μg/kg (n = 25)†	2.25 μg/kg (n = 6)	3.0 μg/kg (n = 12)
AUC_{0-t} (h*ng/mL), mean (SD)‡	11.6 (7.9)	59.6 (97.2)	74.1 (60.6)	131 (139)	148 (122)
C_{max} (ng/mL), mean (SD)§	0.255 (0.238)	0.927 (1.33)	0.978 (0.810)	1.42 (1.68)	1.69 (1.31)

Abbreviation: SD, standard deviation.

*One patient was excluded because of AUC_{0-t} and C_{max} values that were inconsistent with the nominal dose administered.

†One patient was excluded because of a lack of pharmacokinetic samples as a result of premature discontinuation of the study.

‡AUC_{0-t} values for some patients were not estimated because of a lack of quantifiable data and were imputed to be one-half of the lowest reported AUC_{0-t} value (6.78 h*ng/mL/2 = 3.39 h*ng/mL).

§C_{max} values below the assay limit of quantification were imputed to be one-half of the limit of quantification (0.0625 ng/mL).

these patients (1.8%); this patient achieved a $\geq$2-log decrease from the baseline in HCV RNA on days 22 and 29.

Two additional treatment-relapse patients had detectable antibodies to PEG-IFN-λ prior to treatment and throughout the study period; neither had a titer increase during the study. One of these patients had neutralizing antibodies and had very low or unmeasurable PEG-IFN-λ serum levels and no antiviral response throughout the study period. The second patient had preexisting but nonneutralizing antibodies to PEG-IFN-λ and had measurable serum levels of PEG-IFN-λ as well as an antiviral response (a 3.81-$\log_{10}$ decrease on day 29).

Discussion

This study was the first designed to assess the safety, tolerability, and efficacy of PEG-IFN-λ in patients chronically infected with genotype 1 HCV. The results indicate that a broad range of PEG-IFN-λ doses exhibit antiviral activity with limited toxicity when it is administered QW, and this supports the hypothesis that PEG-IFN-λ may be a useful agent in future HCV treatment regimens

SVR, the most definitive measure of antiviral activity, was not evaluated in this study because patients received only 4 weeks of therapy with PEG-IFN-λ with or without RBV. However, relationships between early viral kinetics and long-term response are well established and add clinical relevance to the 4-week antiviral results observed in the current study. Predictive markers of response, including rapid virological response (defined as undetectable HCV RNA at week 4) and early virological response (defined as a >2-$\log_{10}$ decrease in HCV RNA by week 12), have been demonstrated to predict response in treatment-naive patients treated with PEG-IFN-α plus RBV and in treatment-relapse patients as well.[17-21] A recent report demonstrated that a $\geq$2-$\log_{10}$ decrease in HCV RNA by week 4 had a positive predictive value of 52% and a negative predictive value of 94% for predicting SVR in treatment-relapse patients receiving PEG-IFN-α in combination with RBV.[19]

Parts 1 and 2 of the study included patients who had previously responded to therapy with PEG-IFN-α, and this allowed a close examination of the safety profile in a group of patients who were expected to demonstrate antiviral activity (proof of concept) within a short treatment period. As anticipated, antiviral activity was observed in the majority of treatment-relapse patients, especially in those who received a dose of PEG-IFN-$\lambda \geq$ 1.5 μg/kg QW with or without RBV,

with 23 of 24 patients (96%) achieving at least a >2-$\log_{10}$ decrease in HCV RNA. Although information on the viral kinetics of the patients' previous responses to IFN-α-based therapies was not collected as part of the study, this magnitude of HCV RNA decrease in the first 4 weeks of therapy indicates clear antiviral activity potentially comparable to that of PEG-IFN-α.

Unambiguous antiviral activity was also observed in the cohort of treatment-naive patients who were all treated with 1.5 μg/kg PEG-IFN-λ QW plus RBV. Six of the seven treatment-naive patients (86%) achieved a >2-$\log_{10}$ decrease in HCV RNA, and two of seven (29%) achieved undetectable HCV RNA; this was an encouraging result despite the small sample size. Historically published rates of >2-$\log_{10}$ decreases in HCV RNA or undetectable HCV RNA after 4 weeks of therapy with PEG-IFN-α plus RBV in treatment-naive patients range from 42% to 67% and from 7.4% to 11.7%, respectively.[3,16,22]

Recent evidence indicates that the viral response to PEG-IFN-α plus RBV may have a genetic basis.[23,24] Single nucleotide polymorphisms upstream from the IL-28B gene on chromosome 19 have been found to be associated with SVR to PEG-IFN-α plus RBV treatment as well as natural clearance of HCV infection.[23-27] These links to IL-28B were initially found through genome-wide association studies, so the mechanism of action was not determined. The connection to PEG-IFN-λ is of interest because IL-28B is also a type III IFN known as IFN-λ3, which shares approximately 70% sequence identity with IFN-λ1 and shares the same receptor with IFN-λ1. The genes for IFN-λ1 (IL-29), IFN-λ2 (IL-28A), and IFN-λ3 (IL-28B) are all located in this same region of chromosome 19. It is possible that the IFN-λ family has unique antiviral effects important to the control of HCV infection, and studies to assess the impact of the different polymorphisms on the antiviral response to PEG-IFN-λ plus RBV are being conducted.

Overall, PEG-IFN-λ, given QW for 4 weeks, was safe and was well tolerated by most patients. Minimal constitutional symptoms or hematologic effects were observed with PEG-IFN-λ as a single agent. As expected, the addition of RBV increased the incidence of fatigue and other constitutional symptoms in treatment-relapse patients. Previously untreated patients experienced a low incidence of adverse events, despite combination therapy with RBV, and this suggested a possible experience effect from previous treatment in the treatment-relapse patients. The majority of significant aminotransferase elevations occurred in patients with high PEG-IFN-λ exposure values, which were

well above the mean for the individual cohorts. Dose reductions were not allowed in the current study. However, because the aminotransferase elevations were reversible and may be related to high exposure, it may be possible in future studies to manage these effects with dose reductions.

Pharmacokinetic data suggest that the relationship between dose and exposure may be linear and that body weight is not an important determinant of exposure. Future studies will explore fixed microgram doses of PEG-IFN-λ; this is expected to be less complicated for self-administration than weight-based dosing. In addition, the use of a ready-to-use formulation in future studies may also reduce the interpatient variability in PEG-IFN-λ exposure estimates.

Only one patient developed a neutralizing antibody to PEG-IFN-λ during the study. Because the antibody was not observed until day 59, 4 weeks after the last dose of PEG-IFN-λ, no conclusions can be drawn regarding a potential effect on the PEG-IFN-λ serum concentration or antiviral activity. The incidence of neutralizing antibody formation with PEG-IFN-α is approximately 2% to 3%, although direct comparisons between different products may not be valid because the observed incidence of antibody positivity is highly dependent on the sensitivity of the assay.[5,6]

These safety and tolerability results observed with the 4-week PEG-IFN-λ treatment compare favorably with the results of published studies of 48-week PEG-IFN-α therapy, in which up to 67% of patients have experienced fatigue, 62% have experienced headache, 56% have experienced myalgia, 48% have experienced rigors, 46% have experienced fever, 43% have experienced nausea, and 40% have experienced insomnia.[3,28] Although direct comparisons with the safety of PEG-IFN-α therapy are not possible because of the 4-week treatment duration in the current study, the incidence of constitutional side effects observed with PEG-IFN-λ therapy appears to be lower. The myelosuppression associated with IFN-α treatment is related to its binding to leukocytes.[29] Although adverse hematologic events may appear after 4 weeks of therapy, the absence of IFN-λ binding to leukocytes may explain why such adverse effects have not yet been observed with PEG-IFN-λ therapy.

Key limitations in the current study include the lack of a direct comparison between PEG-IFN-λ and PEG-IFN-α, and this restricts any conclusions regarding the relative effects of one agent with respect to the other. Because the current study encompassed only 4 weeks of dosing, the long-term effects of PEG-IFN-λ could not be determined. The open-label design of the study

may have influenced adverse event reporting. Lastly, the study included only patients with genotype 1 HCV infection, and it is not known if these results will translate to patients with other genotypes. Future studies will explore these questions.

The findings from this study offer evidence that PEG-IFN-λ, given QW for 4 weeks, exhibits potent antiviral effects against HCV with the potential for an improved tolerability profile with respect to that traditionally observed for PEG-IFN-α. These results are being tested in larger randomized controlled trials enrolling treatment-naive patients.

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