

# Final Results of A Double-Blind, Placebo-Controlled Trial of the Antifibrotic Efficacy of Interferon- $\gamma$ 1b in Chronic Hepatitis C Patients with Advanced Fibrosis or Cirrhosis

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Interferon- $\gamma$ 1b (IFN- $\gamma$ 1b) is a pleiotropic cytokine that displays antifibrotic, antiviral, and antiproliferative activity. A total of 502 patients with compensated liver disease and an Ishak fibrosis score of 4–6 were randomized in a double-blind, placebo-controlled study, and 488 of these patients received subcutaneous injections of IFN- $\gamma$ 1b 100  $\mu$ g (group 1, n = 169), IFN- $\gamma$ 1b 200  $\mu$ g (group 2, n = 157), or placebo (group 3, n = 162) 3 times a week for 48 weeks. Most patients (83.6%) had cirrhosis at baseline (Ishak score = 5 or 6). Posttreatment liver biopsies were assessed in a blinded fashion for a reduction of 1 or more Ishak points (primary endpoint). Four hundred twenty patients with pretreatment and posttreatment liver biopsies were evaluable and showed no improvement in Ishak score between the 3 treatment groups (12.1%, 12.4%, and 16% of patients in groups 1, 2, and 3, respectively;  $P > 0.05$ ). Analysis of IFN- $\gamma$ -inducible biomarkers revealed that interferon-inducible T cell- $\alpha$  chemoattractant (ITAC), an IFN- $\gamma$ -inducible CXCR3 chemokine was an independent predictor of stable or improving Ishak score. IFN- $\gamma$ 1b was well tolerated. There were similar numbers of deaths in all 3 arms (5, 5, and 4, respectively), and most were related to complications of cirrhosis. **Conclusion:** IFN- $\gamma$ 1b therapy was not able to reverse fibrosis in patients with advanced liver disease for 1 year. Subgroups of patients with elevated ITAC levels and perhaps less advanced disease may be considered for future studies with IFN- $\gamma$ 1b. (HEPATOLOGY 2007;45:569-578.)

Fibrosis is currently viewed as a dynamic rather than a static process; extracellular matrix is constantly being laid down and resorbed, and the progressive accumulation of fibrous tissue is believed to represent a relative im-

balance between profibrotic and antifibrotic processes.<sup>1</sup> The central cells involved in the pathogenesis of hepatic fibrosis are hepatic stellate cells (HSCs), also known as lipocytes, fat-storing cells, Ito cells, or myofibroblasts.<sup>2</sup> Cytokines play major roles in all stages in the development of fibrosis, including hepatocyte injury, inflammatory response, altered function of sinusoidal cells (particularly HSCs), extracellular matrix accumulation, and matrix degradation. Cytokines play an especially important role in perpetuating and modulating the effects of activated HSCs.

In experimental models of fibrosis, transforming growth factor- $\beta$  (TGF- $\beta$ ) has been shown to play a key role in stimulating and maintaining the fibrogenic process. In the liver, TGF- $\beta$  stimulates the expression of extracellular matrix proteins and collagen, inhibits collagenases, and promotes the activation of fat-storing HSCs toward a myofibroblast phenotype.<sup>3</sup> In addition, inhibition of TGF- $\beta$  is effective in preventing fibrosis as well as in preserving organ function.<sup>1</sup>

Interferon gamma (IFN- $\gamma$ ) has been shown to be a key counter-regulatory antifibrotic cytokine down-regulating the activity of TGF- $\beta$ .<sup>4</sup> IFN- $\gamma$  inhibits the interaction of downstream proteins that are normally activated after the binding of TGF- $\beta$  to its cellular receptor. As a result,

Abbreviations: HSC, hepatic stellate cell; IFN- $\gamma$ 1b, interferon- $\gamma$ 1b; IP, interferon-inducible protein; ITAC, interferon-inducible T cell- $\alpha$  chemoattractant; MIG, monokine induced by interferon- $\gamma$ ; TGF- $\beta$ , transforming growth factor- $\beta$ .

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transcriptional responses to TGF- $\beta$  signaling are inhibited. The degree of inhibition depends in part on the relative amounts of TGF- $\beta$  and IFN- $\gamma$ , indicating that the extent of inhibition (or activation) of TGF- $\beta$  responsive genes may be determined by the balance of TGF- $\beta$  and IFN- $\gamma$  signals. This provides a theoretical rationale for the use of pharmacological doses of IFN- $\gamma$  even in those settings in which there are elevated levels of intrinsic IFN- $\gamma$  and TGF- $\beta$ .<sup>5</sup>

Experimental data from *in vitro* studies, studies in animal models of liver fibrosis and studies in humans with idiopathic pulmonary fibrosis all support a potential therapeutic role for IFN- $\gamma$ 1b in the inhibition of fibrosis in the liver and other organs.<sup>1-4,6-8</sup> Studies of the *in vitro* effects of IFN- $\gamma$  on cultured murine as well as human HSCs consistently demonstrate that IFN- $\gamma$  inhibits the proliferation and culture-induced activation of these cells with resultant inhibition of the expression of messenger RNA encoding extracellular matrix proteins, leading to a significant reduction in the production of extracellular matrix proteins.<sup>2-4</sup> In addition, several studies have been published on the antifibrotic activity of IFN- $\gamma$  in animal models of liver fibrosis. These studies consistently demonstrate that IFN- $\gamma$  administered during the period of hepatic injury is capable of reducing the overall mass of extracellular matrix and of reducing the degree of histologically evident fibrosis present in the liver.

A small pilot study by Saez-Royuela et al.<sup>7</sup> randomized 30 adults with chronic hepatitis C and persistently elevated serum ALT levels to either IFN- $\gamma$ 2c or IFN- $\gamma$ 1b. There was a trend toward a decrease in the fibrosis score in IFN- $\gamma$ 1b recipients with a reduction in fibrosis from  $1.2 \pm 1.0$  to  $0.7 \pm 1.0$  on the Knodell fibrosis score. Another pilot trial of 14 patients who had failed prior treatment evaluated IFN- $\gamma$ 1b in doses of 100, 200, or 400  $\mu$ g subcutaneously (SC) three times a week for 4 weeks. Although relatively well tolerated, IFN- $\gamma$ 1b had no effect on HCV RNA levels. Fibrosis was not assessed. These experimental and clinical data justified the study of IFN- $\gamma$ 1b in a phase 2b study to evaluate its role as an antifibrotic agent.

## Patients and Methods

This study was designed as a double-blind, randomized, placebo-controlled, prospective, multicenter, 3-arm study comparing 2 doses of IFN- $\gamma$ 1b with placebo. IFN- $\gamma$ 1b (100 or 200  $\mu$ g) or placebo was administered 3 times a week via subcutaneous injection for 48 weeks with a 4-week follow-up period. Patients were followed for an additional 4 weeks after completing treatment (or after posttreatment liver biopsy if the patient discontinued early) for evidence of hepatic decompensation and laboratory evaluations.

**Patient Selection.** The study population consisted of patients with advanced liver fibrosis or cirrhosis (Ishak fibrosis stage 4, 5, or 6) without clinical decompensation due to chronic hepatitis C virus infection and who were viremic. Patients had to have a history of prior treatment with IFN- $\alpha$ -based therapy, a contraindication to, or be deemed unlikely to benefit from therapy with IFN- $\alpha$  or pegylated IFN- $\alpha$ .

Patients included in this trial were men or women age 18 to 75 years with chronic hepatitis C infection based on a history of positive anti-HCV antibody and/or HCV RNA. Patients were required to have a positive HCV RNA via branched DNA/transcription-mediated amplification (bDNA/TMA) assay at screening, an adequate liver biopsy specimen as deemed by the central pathologist obtained either within 12 months of screening or at the time of screening, demonstrate a willingness to undergo a liver biopsy at the beginning (if required) and end of treatment, and have stage 4, 5, or 6 liver fibrosis according to the Ishak scoring system.

Patients with any of the following were excluded from randomization: presence of clinically evident ascites requiring active diuretic therapy; history of or therapy for hepatic encephalopathy; history of gastroesophageal variceal bleeding within the previous 2 years; platelet count  $<60,000/\text{mm}^3$ ; serum ALT level  $>10$  times the upper limit of normal; alpha-fetoprotein level  $>200$  ng/ml or alpha-fetoprotein level between 50-200 ng/ml in association with liver ultrasound or other radiographic abnormality suspicious for hepatic neoplasm; serum creatinine level  $>1.6$  mg/dl; hematology outside of specified limits (neutrophil count  $<1000/\text{mm}^3$ , hemoglobin  $<10$  g/dl in males and  $<9$  g/dl in females); unstable or uncontrolled thyroid disease; treatment with any IFN- $\alpha$  within the previous 8 weeks; presence of clinically significant cryoglobulinemia, autoimmune hepatitis, alpha-1 antitrypsin deficiency, hemochromatosis, Wilson's disease, drug-induced or toxin-induced liver disease, alcohol-related liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, chronic hepatitis B, or detectable HBsAg; HIV antibody-positive, concurrent therapy with immunosuppressive drugs or cytotoxic agents; behavior suggesting a significant risk of poor compliance; pre-existing (within the previous 2 years) or active psychiatric condition including severe depression, major psychoses, suicidal ideation, or suicidal attempts; pregnancy or lactation; or treatment with any investigational therapy for any indication within 28 days before treatment.

**Study Design and Assessments.** Potential patients underwent a screening medical history, physical examination, and laboratory tests. A prestudy liver biopsy was obtained and evaluated by a central pathologist to

determine if patients met the required histological inclusion criteria (adequate baseline liver biopsy specimen and appropriate degree of liver fibrosis). Randomization was stratified for patients with advanced fibrosis (Ishak stage 4) versus cirrhosis (Ishak stage 5 or 6) and by investigator center. All patients were seen and evaluated at baseline and at weeks 1, 4, 8, 16, 24, 36, 42, 48, and 52. At the end of treatment (week 48), patients had a follow-up liver biopsy. Four weeks after taking the final dose of study drug, patients returned to the clinic for the week 52 follow-up evaluation. Collection of all serious adverse event data was to continue during an additional 48 weeks of posttherapy follow-up. At the conclusion of the study, patients assigned to placebo therapy who completed the study, were compliant, and underwent the posttreatment liver biopsy were eligible to receive IFN- $\gamma$ 1b in a follow-up study if data from this study indicated that the drug was safe and effective. Informed consent was obtained from each patient included in the study, and the protocol followed the ethical guidelines of the Declaration of Helsinki as reflected in *a priori* approval each institution's Human Research Committee.

The primary objective of this study was to evaluate the proportion of patients showing a reduction of 1 or more points on the fibrosis staging score (using the Ishak staging system) on liver biopsy after treatment with one of two dose levels of IFN- $\gamma$ 1b for 48 weeks compared with placebo recipients. The primary quantitative measure of liver fibrosis was a blinded assessment by one pathologist (Z. G.).

The secondary efficacy objectives of the study were multiple: to evaluate the change in liver fibrosis histology score as graded by the METAVIR, Knodell, and Laennec fibrosis staging systems; to evaluate the relative change in fibrosis based on a comparison of paired prestudy and poststudy liver biopsies (blinded to order) based on an assessment of improved, unchanged, or worsened; to evaluate the change in the necroinflammatory component of the Knodell Histologic Activity Index; to evaluate the change in HCV RNA viral load before, during, and at the end of therapy; to evaluate the proportion of patients developing clinical manifestations of hepatic decompensation, including ascites, gastrointestinal bleeding due to varices, spontaneous bacterial peritonitis, hepatic encephalopathy, hepatocellular carcinoma, or death due to liver cirrhosis; to evaluate the change in laboratory indices of liver function (serum albumin, prothrombin time, bilirubin, and platelet count); and, lastly, to evaluate the change in portal hemodynamics in a subset of patients at a subset of centers.

Additional secondary measures included the semi-automated morphometric quantitation of collagen content in liver biopsy specimens following Sirius Red staining. Hepatitis C RNA levels were assessed via quantitative HCV RNA branched DNA assay supplemented by transcription-mediated amplification assay. Two commercially available bioassays previously validated for liver fibrosis in HCV, serum hyaluronic acid (Corgenix, Denver, CO), and YKL-40 (Metra Biosystems, Mountain View, CA) were performed. These ELISAs were performed on serum at baseline, at 24 weeks, and at the end of therapy. Serum specimens were centrifuged and frozen at each blood draw to be analyzed later for serum interferon-inducible T cell- $\alpha$  chemoattractant (ITAC), monokine induced by IFN- $\gamma$  (MIG)-9, and interferon-inducible protein (IP)-10 markers of IFN- $\gamma$  activity. All liver biopsies were judged for adequacy, length, and fragmentation and were graded 0-4 based on percentage of steatosis present (0 = none; 1+ = >0%-5%; 2+ = 6%-33%; 3+ = 34%-66%; 4+ = >66%).

The safety objectives of the trial were to evaluate the safety and tolerability of IFN- $\gamma$ 1b (100  $\mu$ g or 200  $\mu$ g 3 times a week) administered subcutaneously for 48 weeks as assessed by clinical signs and symptoms and laboratory measures and to evaluate for the presence of anti-IFN- $\gamma$ 1b antibodies. An independent data safety monitoring board was formed to monitor safety. An interim assessment of safety and tolerability was performed after the first 90 patients had completed 8 weeks of therapy; thereafter, the data safety monitoring board met approximately twice yearly. All adverse events were assessed by the investigator with regard to seriousness, severity, grade (according to the modified WHO common toxicity criteria), and relationship to study drug. All clinical laboratory values were compared with baseline values, and any change was tabulated separately. Results of the hemodynamic measurements and morphometric collagen studies are reported separately.

**Statistical Analysis.** The sample size of 500 patients (approximately 167 per group) was selected to be sufficient to detect a difference of 20% between treatments (e.g., 40% in an IFN- $\gamma$ 1b treatment arm versus 20% in the placebo arm) in the proportion of patients with at least a 1-point difference in the Ishak liver fibrosis score with a power of 0.93 in the primary analysis. This calculation assumed that up to 20% of patients would not have paired liver biopsies.

The primary efficacy analysis compared the proportion of patients showing a reduction with pretreatment of 1 or more points on the Ishak fibrosis staging score at the week

48 liver biopsy between each IFN- $\gamma$ 1b dose and placebo (pairwise comparisons) using the Cochran-Mantel-Haenszel test stratified by patient disease status at entry (advanced fibrosis or cirrhosis). The primary analysis included all patients randomized with intention to treat who received any study drug (modified intent-to-treat population). Patients without a second biopsy were considered to have no change.

A secondary analysis of the primary efficacy variable was conducted on all patients randomized with intention to treat. Analyses of secondary efficacy measures were performed using the modified intent-to-treat cohort of patients for clinical end points and the modified intent-to-treat cohort of patients who had a posttreatment liver biopsy for histological end points. Cochran-Mantel-Haenszel methods were employed for categorical outcomes. ANOVA methods were used for continuous numerical outcomes. Missing values were not imputed for any variables, meaning that all missing values were treated as missing data.

The percent change from baseline to week 24 in ITAC, MIG-9, and IP-10 levels were compared pairwise among the treatment groups using the Wilcoxon rank-sum test. This time point was chosen based on analysis of a small number of samples showing a peak of cytokine induction response occurring by week 24 of therapy.

Logistic regression was used to measure the impact of the percent change in each biomarker score in predicting absence of worsening in Ishak fibrosis scores from baseline to end of study. Significance was determined with Wald's chi-square statistic.

Percentages of patients with a  $\geq 60\%$  change in ITAC induction were calculated using the total number of patients with paired biopsy data in each treatment group. The optimal cutoff point of 60% change in ITAC induction was determined by maximizing Cohen's kappa based on using all observed percent changes in ITAC values between the 25th and 75th percentiles as potential cut points.

Fisher's exact test was used to compare the treatment groups in terms of patients with percent ITAC induction greater than the optimal ITAC induction point, and to compare patients above and below the optimal ITAC induction point in terms of worsening Ishak fibrosis scores. Logistic regression was also used to measure the impact of IFN- $\gamma$  dose in predicting nonworsening Ishak fibrosis scores, with observations weighted by percent ITAC induction. Significance was determined with Wald's chi-square statistic. The value of the model was measured with log likelihood statistics.

## Results

### *Patient Disposition and Demographics*

During the study, 502 patients were randomized at 73 sites, and 488 patients received at least one dose of study drug; 78% of patients received  $\geq 80\%$  of scheduled doses. Figure 1 shows the patient disposition.

The mean age was 51.7 years (range, 34-76 years). Most patients were male (69%) and Caucasian (70%); the distribution was approximately equal among treatment groups. The only statistically significant difference between groups was in the numbers of males and females in the 100- $\mu$ g IFN- $\gamma$ 1b group and the placebo group ( $P = 0.030$ ).

The majority of patients (84%) had cirrhosis at baseline: 15% had Ishak stage 4 fibrosis and 84% had Ishak stage 5 or 6 fibrosis. Table 1 presents patient demographics and selected baseline characteristics, and Table 2 presents baseline Ishak scores and hepatitis C history. The mean length of 490 of the needle biopsies was 21 mm with standard deviation of 8 mm.

### *Primary Efficacy Analysis*

There were no statistically significant differences among treatment groups in the proportion of patients who showed a reduction (i.e., improvement) of 1 or more points on the Ishak fibrosis score on liver biopsy at week 48 compared with baseline (Fig. 2).

### *Additional Efficacy End Points*

**Worsening Fibrosis.** Percentages of patients who showed worsening of 1 or more points or no change on the Ishak fibrosis scale were similar across treatment groups. Mean changes from baseline to final measurement in Ishak fibrosis score were minimal in each of the 3 treatment groups. Baseline steatosis and impact on changes from baseline in fibrosis were analyzed to determine if there was a correlation present based on previously published data. No correlation was detected (Table 3). There was no correlation of the biomarkers hyaluronic acid and YKL-40 with change in fibrosis on liver biopsy. This data will be more fully discussed in a separate manuscript.

**Changes in Levels of ITAC, MIG-9, and IP-10.** Changes in levels of serum ITAC, MIG-9, and IP-10 markers of IFN- $\gamma$  activity at week 24 were analyzed and compared with baseline. The percent change in levels of MIG-9, IP-10, and ITAC from baseline to week 24 was significantly higher in each IFN- $\gamma$ 1b group compared with placebo ( $P < 0.001$  in all cases). The mean percent changes from baseline to week 24 in ITAC levels were significantly higher in the 100- $\mu$ g IFN- $\gamma$ 1b and the 200- $\mu$ g IFN- $\gamma$ 1b group than in the placebo group

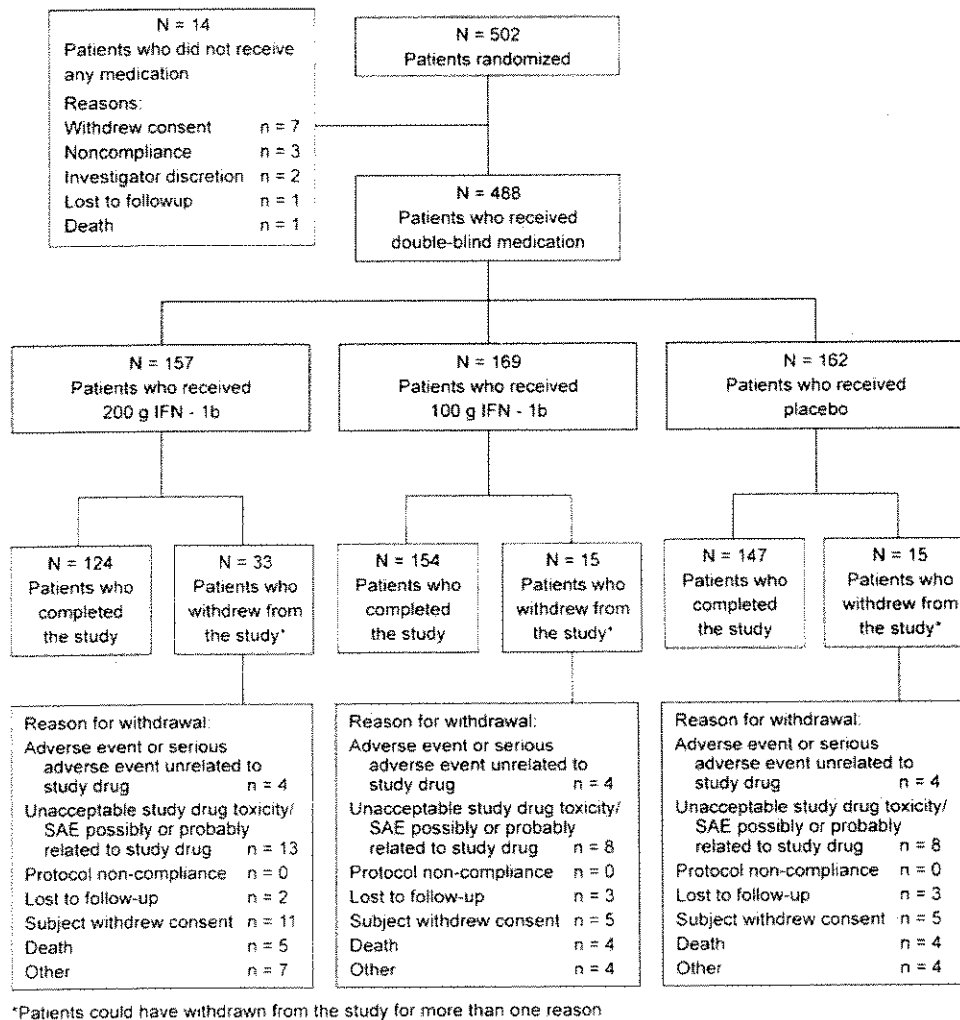


Fig. 1. Patient disposition.

[56.4% ± 106.2, 83.9% ± 115.6, 0.5% ± 41.9, respectively ( $P < 0.001$  for both comparisons)] and the mean percent change in ITAC levels in the 200- $\mu$ g IFN- $\gamma$ 1b group was significantly higher than in the 100- $\mu$ g IFN- $\gamma$ 1b group ( $P = 0.0279$ ).

The mean percent changes from baseline to week 24 in levels of MIG-9 were significantly higher in the 100- $\mu$ g IFN- $\gamma$ 1b and the 200- $\mu$ g IFN- $\gamma$ 1b group than in the placebo group (50.2% ± 102.3, 110.2% ± 130.5, and -7.8% ± 31.8, respectively [ $P < 0.001$  for both comparisons]). The mean percent change in MIG-9 levels in the 200- $\mu$ g IFN- $\gamma$ 1b treatment group was significantly higher than in the 100- $\mu$ g IFN- $\gamma$ 1b treatment group ( $P \leq 0.001$ ).

The mean percent changes from baseline to week 24 in levels of IP-10 were significantly higher in the 100- $\mu$ g IFN- $\gamma$ 1b and the 200- $\mu$ g IFN- $\gamma$ 1b group than in the placebo group (50.1% ± 80.8, 67.7% ± 87.8, and 5.4% ± 40.6, respectively [ $P < 0.001$  for both comparisons]),

**Table 1. Demographics and Baseline Characteristics (MITT Population, N = 488)**

Characteristic	IFN-g 1b		
	200 mg (N = 157) n (%)	100 mg (N = 169) n (%)	Placebo (N = 162) n (%)
Age at screening (years), mean (range)	51.5 (36-76)	52.1 (34-73)	51.6 (34-71)
Sex <sup>a</sup> , n (%)			
Female	43 (27)	65 (38)	44 (27)
Male	114 (73)	104 (62)	118 (73)
Race/ethnicity, n (%)			
Caucasian	114 (73)	122 (72)	108 (67)
African American	12 (8)	20 (12)	18 (11)
Asian/Pacific Islander	2 (1)	2 (1)	3 (2)
Other <sup>b</sup>	3 (2)	6 (4)	4 (2)
Body mass index, mean (SD)	29.7 (4.8)	29.9 (6.0)	30.4 (5.4)

<sup>a</sup>The difference between the number of males and females in the 100- $\mu$ g IFN- $\gamma$  1b group and in the placebo group was statistically significant ( $P = 0.030$ ). This was the only statistically significant difference between groups.

<sup>b</sup>Other includes Asian/Pacific Islander, Filipino, Indian, Middle Eastern, Native American/ Alaskan, Spanish, and Sudanese.

**Table 2. Baseline Ishak Score and Hepatitis C History (MITT Population, N = 488)**

Characteristic	IFN- $\gamma$ 1b		
	200 $\mu$ g (N = 157) n (%)	100 $\mu$ g (N = 169) n (%)	Placebo (N = 162) n (%)
Baseline Ishak score			
4 Marked bridging	21 (13)	32 (19)	21 (13)
5 Incomplete cirrhosis	52 (33)	48 (28)	44 (27)
6 Established cirrhosis	84 (54)	88 (52)	92 (57)
Probable mode of transmission			
IV drug use	54 (34)	62 (37)	51 (31)
Blood transfusion	45 (29)	62 (37)	56 (35)
Sexual transmission	3 (2)	4 (2)	1 (1)
Other (including unknown)	55 (35)	41 (24)	54 (33)
Baseline mean HCV RNA levels (RNA copies/ml)	n = 156 2,679,509	n = 169 2,911,059	n = 161 2,698,073
HCV genotype result			
1 (1a or 1b)	140 (90)	141 (84)	139 (86)
2	4 (3)	10 (6)	7 (4)
3	7 (4)	8 (5)	5 (3)
Mixed, other, or missing	6 (4)	10 (6)	11 (7)

NOTE. Percentages are based on the number of patients in that treatment group.

but the mean percent changes in IP-10 in the IFN- $\gamma$ 1b groups were not significantly different from each other ( $P = .1370$ ).

**Association of Marker Expression and Change in Liver Histology.** Logistic regression analysis showed that induction of ITAC (percent change in ITAC levels at week 24 from baseline) was associated with an absence of worsening in liver histology using the Ishak fibrosis score [Wald chi-square statistic = 6.36 ( $P = 0.017$ )] (Fig. 3). In contrast, neither MIG-9 induction nor IP-10 induction at week 24 compared with baseline was associated with absence of worsening or with stable liver histology using the Ishak fibrosis score [Wald chi-square statistic = 2.41 ( $P = .1203$ ), Wald chi-square statistic = 1.81 ( $P = .1777$ ), respectively].

**IFN- $\gamma$ 1b Treatment and Expression of ITAC.** Significantly more patients treated with either dose of IFN- $\gamma$ 1b had serum levels of ITAC  $\geq 59.3\%$  compared with patients receiving placebo ( $P < 0.0001$  for both comparisons). There was a significantly higher percentage of patients with  $\geq 60\%$  change in ITAC induction at week 24 from baseline in the 100- $\mu$ g IFN- $\gamma$ 1b treatment group versus placebo [30 patients (30/91, 33.0%) versus 4 patients (4/90, 4.4%);  $P < 0.001$ ] and the 200- $\mu$ g IFN- $\gamma$ 1b treatment group versus placebo [34 patients (34/87, 39.1%) versus 4 patients (4/90, 4.4%);  $P < 0.001$ ]. However, the numbers of patients who had an increase in ITAC levels of  $\geq 59.3\%$  in the treatment groups (33% versus 39% for 100- $\mu$ g versus 200- $\mu$ g IFN- $\gamma$ 1b groups,

respectively) were not significantly different from each other ( $P = .4366$ ).

**ITAC Expression and Histology.** Patients who had  $\geq 60\%$  induction of ITAC had a significantly better liver histology outcome compared with patients with  $< 60\%$  induction of ITAC. Only 2 of the 62 patients (3.2%) with  $\geq 60\%$  induction of ITAC experienced worsening Ishak scores compared with 27 of the 177 patients (15.3%) with  $< 60\%$  ITAC induction ( $P = 0.0118$ ).

**IFN- $\gamma$ 1b Treatment and Change in Liver Histology.** Treatment with either 100  $\mu$ g or 200  $\mu$ g IFN- $\gamma$ 1b three times a week is associated with absence of worsening in liver histology when weighted for ITAC induction [Wald chi-square statistic = 161.8 ( $P < 0.0001$ ) for 100- $\mu$ g IFN- $\gamma$ 1b treatment, Wald chi-square statistic = 8.3 ( $P = 0.0039$ ) for 200- $\mu$ g IFN- $\gamma$ 1b treatment, respectively].

**IFN- $\gamma$ 1b Treatment and Change in HCV RNA Levels.** Five patients in the IFN- $\gamma$ 1b groups and none in the placebo group had undetectable HCV RNA via transcription-mediated amplification while on treatment. In 4 of 5 patients, 3 of whom were not adherent to therapy or required prolonged dose reduction, viremia recurred while on IFN- $\gamma$ 1b therapy (Table 4).

### Safety

IFN- $\gamma$ 1b therapy was generally well tolerated. Common treatment-related adverse events that occurred more frequently in the IFN- $\gamma$ 1b groups than in the placebo group were those expected with IFN therapy, and included (in decreasing order of occurrence) headache, fa-

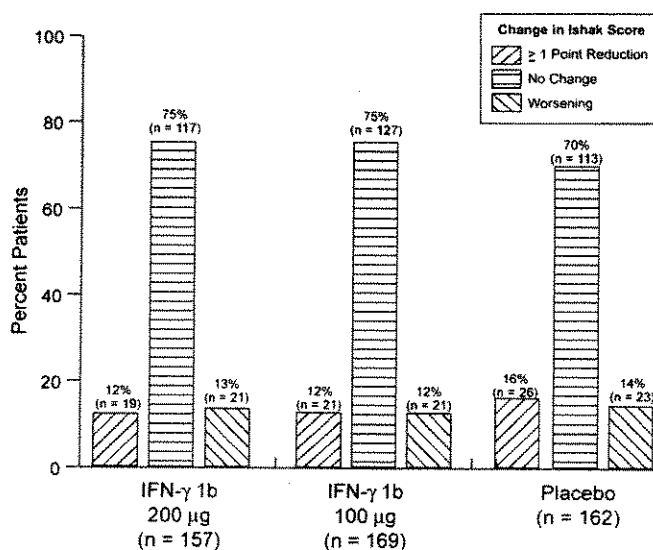


Fig. 2. Primary end point: proportion of patients showing reduction of  $\geq 1$  point, no change, or worsening in Ishak fibrosis staging score on liver biopsy at 48 weeks compared with baseline (modified intent-to-treat population, n = 488).

**Table 3. Baseline Steatosis and Impact on Changes from Baseline in Ishak Fibrosis Scores Modified Intent-to-Treat Patient Population**

Variable	IFN-200	IFN-100	Placebo	Total	P Value
Number of Patients	87	91	90	268	
None	18 (20.7%)	19 (20.9%)	19 (21.1%)	56 (20.9%)	
1+	46 (52.9%)	52 (57.1%)	45 (50.0%)	143 (53.4%)	
2+	21 (24.1%)	20 (22.0%)	22 (24.4%)	63 (23.5%)	
3+	2 (2.3%)	0 (0.0%)	4 (4.4%)	6 (2.2%)	
Absent	18 (20.7%)	18 (20.9%)	19 (21.1%)	56 (20.9%)	0.9976
Present	69 (79.3%)	72 (79.1%)	71 (78.9%)	212 (79.1%)	
Change in Ishak Fibrosis Score					
All Patients					
Number of patients	71	86	82	239	
No Improvement	61 (85.9%)	77 (89.5%)	66 (80.5%)	204 (85.4%)	0.2511
Improvement	10 (14.1%)	9 (10.5%)	16 (19.5%)	35 (14.6%)	
Patients with steatosis absent at baseline					
Number of patients	11	18	17	46	
No Improvement	9 (81.8%)	16 (88.9%)	12 (70.6%)	37 (80.4%)	0.3885
Improvement	2 (18.2%)	2 (11.1%)	5 (29.4%)	9 (19.6%)	
Patients with steatosis present at baseline					
Number of patients	60	68	65	193	
No Improvement	52 (86.7%)	61 (89.7%)	54 (83.1%)	167 (86.5%)	0.5335
Improvement	8 (13.3%)	7 (10.3%)	11 (16.9%)	26 (13.5%)	

NOTE. The P values are from the likelihood ratio chi-square test.

tigue, rigors, myalgia, arthralgia, pyrexia, influenza-like illness, pain, and muscle cramps.

Clinically relevant adverse events are listed in Table 5, as are the number of patients with dose reductions due to adverse events and the number of patients who discontinued treatment due to adverse events.

Fifteen patients died during the study. Four of 162 (2.5%) patients in the placebo group and 11 of 326 (3.4%)

patients in the IFN- $\gamma$ 1b treatment groups died. There were no statistically significant differences in the numbers of deaths among patients in the 3 groups [ $P = 0.8048$  (two-sided Fisher exact test)]. Most deaths were due to liver disease (Table 6). Clinical manifestations of decompensation were present in 9% to 13% of patients in all 3 treatment groups and were not significantly different between placebo and IFN- $\gamma$ 1b treatment groups (Table 7).

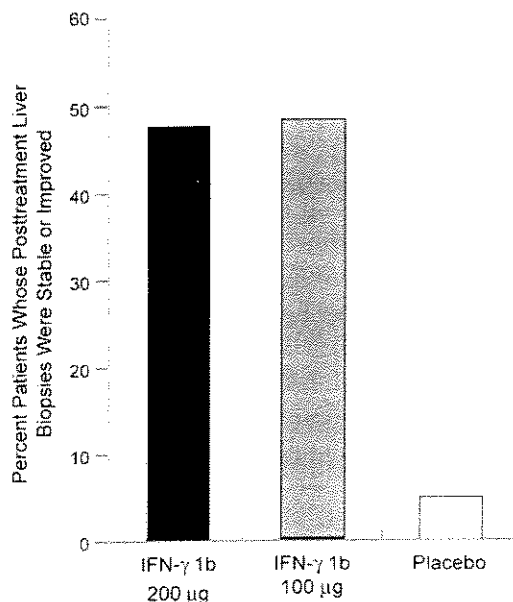


Fig. 3. Percent of patients with  $\geq 59\%$  ITAC induction whose post-treatment liver histology improved or did not change (histologically evaluable modified intent-to-treat population with available week 24 ITAC result,  $n = 268$ ).

## Discussion

This study demonstrates that IFN- $\gamma$ 1b as monotherapy for 48 weeks is not effective at reversing advanced fibrosis or cirrhosis in patients with chronic hepatitis C. IFN- $\gamma$ 1b appears to be well tolerated in most patients at the doses that were evaluated in this trial (100  $\mu$ g and 200  $\mu$ g thrice weekly). Antiviral efficacy at 400  $\mu$ g three times a week did not appear to be greater in a pilot trial, and adverse events were more frequent and severe,<sup>8</sup> suggesting that the dose range studied in this trial was adequate.

Two earlier studies provided data relevant to assessing the potential antifibrotic effects of IFN- $\gamma$ 1b in humans. The first was a randomized controlled study on the effects of IFN- $\gamma$ 1b in idiopathic pulmonary fibrosis, a progressive fibrosing disease of unknown etiology with a mean survival time of 2 to 4 years. Ziesche et al.<sup>6</sup> conducted an open-label, randomized, controlled trial comparing the safety and efficacy of IFN- $\gamma$ 1b plus low-dose prednisolone ( $n = 9$ ) with that of prednisolone alone ( $n = 9$ ) in patients with idiopathic pulmonary fibrosis (confirmed via biopsy or via high-resolution CT). Over the course of

**Table 4. Patients Who Achieved Undetectable HCV RNA**

Patient	Dose	Screening	HCV RNA (IU/ml)			Notes
			Week 4	Week 24	Week 48	
16-007	100 $\mu$ g	46328		<615	883836	Completed treatment through Week 48
20-0222	100 $\mu$ g	259980			<615	Dose held at Week 3 and 4, then resumed treatment at half dose through Week 48
30-012	200 $\mu$ g	995022	<615	738945		Completed treatment through Week 48
48-014	200 $\mu$ g	11757	<615	2373		Completed treatment through Week 48, skipped 1 dose at Week 43
31-031	100 $\mu$ g	412140	-	<615	163263	Completed treatment through Week 48 but became noncompliant and skipped doses from Week 29 through Week 48

the study, all patients treated with IFN- $\gamma$ 1b showed improvement in pulmonary function. In contrast, patients treated with prednisolone alone showed deterioration in their condition.

The second study was the pilot trial by Saez-Royuela et al.<sup>7</sup> evaluating the effect of IFN- $\gamma$ 1b on fibrosis in HCV. A more recent study by Weng et al.<sup>9</sup> showed that in HBV, IFN- $\gamma$  therapy for 9 months significantly improved fibrosis scores ( $P = 0.0001$ ). This study was performed in a much smaller cohort of patients with a different disease (HBV rather than HCV).

There are several factors that could have adversely affected a positive outcome in our study. The study did not explore the impact of longer durations of therapy with or without concomitant HCV antiviral therapy on liver fibrosis nor the impact of IFN- $\gamma$ 1b therapy on early-stage fibrosis. Because our study was the first to use fibrosis score on liver biopsy as the primary endpoint for treatment rather than ALT, viral load, and histology, we took a number of risks that may have resulted in failure. In retrospect, we question whether the proper patient population was selected. It may be too difficult to show a response in a population with cirrhosis, because these patients have roughly 15 times more collagen deposition than those with bridging fibrosis.<sup>3</sup> Perhaps the drug would have shown benefit in patients with F1-3, rather than such late-stage disease. The adequacy of the duration of therapy must also be questioned. A longer duration of therapy, perhaps 2 years or longer, may be required to impact liver fibrosis. The HALT-C, Copilot, and EPIC trials are all designed to analyze effects after 3-5 years of therapy in advanced liver disease.<sup>10-12</sup>

Was the dose adequate? The dose was selected based on the findings of the idiopathic pulmonary fibrosis trials and doses of IFN- $\gamma$ 1b used in the treatment of chronic granulomatous disease. It is unlikely that patients would have tolerated higher dose levels based on constitutional toxicity and the effects on neutrophils and hematocrit seen at the 400  $\mu$ g dose level in the Soza et al. trial.<sup>8</sup> Their

findings suggest that even if higher doses were effective, they probably would not be clinically tolerated. Our trial showed discontinuations of 11% and 14% overall in the 200  $\mu$ g and 100  $\mu$ g groups, respectively, as opposed to 6% in the placebo group. These are acceptable discontinuation rates and are comparable to pegylated IFN studies,<sup>13-15</sup> but higher rates would not be acceptable.

The risk of underestimation of fibrosis via liver biopsy is significant.<sup>16</sup> However, the chance of underestimation is reduced dramatically by using paired liver biopsies. Furthermore, the effect of underestimation should be the same in the treatment and placebo groups. The effects of steatosis on antifibrotic effects of a drug are unknown; however, there is clear evidence that steatosis worsens fibrosis<sup>17,18</sup> and interferes with the effectiveness of IFN- $\alpha$  therapies.<sup>18-20</sup> It is plausible that steatosis might interfere with the antifibrotic effects of IFN- $\gamma$ 1b.

The rate of hepatic decompensation in all 3 groups, including placebo, was greater than one might expect based on the Fattovich data for HCV. In our study, we saw a 9% rate of decompensation over 1 year, whereas the Fattovich data would suggest a rate closer to 3%-4%.<sup>21</sup> These higher rates are likely a reflection of the rigid enrollment criteria based on adequate biopsies, lessening the probability of underestimation of fibrosis stage on biopsy and due to the higher weights and steatosis stage of U.S. patients compared with European patients in the Fattovich trial, both of which are independent predictors of progression. The HALT-C trial rates of progression are similar after 1 year.<sup>10</sup> Portal hypertension outcomes were not significant and will be presented in a separate manuscript.

There were 5 patients with a transient decrease in HCV RNA to undetectable levels, so perhaps there is a modest antiviral effect in a small percentage of patients. These results do not suggest there would be synergistic antiviral effects of IFN- $\alpha$  and IFN- $\gamma$ 1b; however, 3 publications suggest synergy exists *in vitro*.<sup>22-24</sup> Furthermore, other synergistic effects, both positive (antifibrotic) and nega-

**Table 5. Incidence of Adverse Events (AEs) and of Dose Reduction, and Drug Discontinuation Due to AEs**

Variables	IFN- $\gamma$ 1b		Placebo (N = 162) n (%)
	200 $\mu$ g (N = 157) n (%)	100 $\mu$ g (N = 169) n (%)	
<b>Adverse Events</b>			
Patients with any AE	156 (99)	168 (99)	161 (99)
Blood and lymphatic disorders	32 (20)	25 (15)	18 (11)
Neutropenia	10 (6)	9 (5)	6 (4)
Grade 3 or 4 neutropenia	4 (3)	4 (3)	0
Thrombocytopenia	7 (4)	4 (2)	7 (4)
Grade 3 or 4 thrombocytopenia	1 (1)	0	2 (1)
Anemia	5 (3)	3 (2)	5 (3)
Grade 3 or 4 anemia	1 (1)	1 (1)	3 (2)
Endocrine disorders	1 (1)	1 (1)	0
Hypothyroidism	1 (1)	0	0
Grade 3 or 4 hypothyroidism	0	0	0
Eye disorders	29 (18)	22 (13)	22 (14)
Gastrointestinal disorders	100 (64)	102 (60)	90 (56)
Nausea	36 (23)	42 (25)	41 (25)
Grade 3 or 4 nausea	1 (1)	2 (1)	0
Diarrhea	24 (15)	24 (14)	31 (19)
Grade 3 or 4 diarrhea	0	1 (1)	0
General disorders and administration site conditions	141 (90)	148 (88)	115 (71)
Fatigue	71 (45)	65 (38)	61 (38)
Grade 3 or 4 fatigue	3 (2)	4 (2)	3 (2)
Pyrexia	54 (34)	52 (31)	21 (13)
Grade 3 or 4 pyrexia	1 (1)	3 (2)	0
Influenza-like illness	35 (22)	32 (19)	23 (14)
Grade 3 or 4 influenza-like illness	2 (1)	0	0
Peripheral edema	19 (12)	30 (18)	18 (11)
Grade 3 or 4 peripheral edema	0	0	1 (1)
Infections and infestations	54 (34)	89 (53)	69 (43)
Musculoskeletal and connective tissues disorders	108 (69)	107 (63)	90 (56)
Myalgia	50 (32)	52 (31)	34 (21)
Grade 3 or 4 myalgia	3 (2)	1 (1)	0
Psychiatric disorders	77 (49)	81 (48)	82 (51)
Depression	22 (14)	27 (16)	32 (20)
Grade 3 or 4 depression	1 (1)	2 (1)	0
Patients with respiratory, thoracic, and mediastinal disorders	67 (43)	72 (43)	67 (41)
<b>Dose reductions due to AEs</b>	12 (8)	8 (5)	4 (3)
<b>Study treatment discontinuations due to AEs</b>	22 (14)	18 (11)	9 (6)
Due to blood and lymphatic disorders	4 (3)	1 (1)	0
Due to general disorders and administration site conditions	6 (4)	4 (2)	3 (2)
Due to musculoskeletal and connective tissues disorders	6 (4)	2 (1)	0
Myalgia	2 (1)	1 (1)	0
Due to psychiatric disorders	7 (4)	2 (1)	3 (2)

tive (hematological or systemic toxicities), could occur. A pilot dose-escalated, phase 2 trial evaluating the safety and tolerability of combination of IFN- $\gamma$ 1b and daily dosing of consensus IFN is currently in progress.

Our study suggests an association between ITAC induction and improvement or no change in liver fibrosis (i.e., no worsening) during IFN- $\gamma$ 1b therapy. The mechanism underlying this association is not clearly understood. However, hypotheses may be derived from data generated in another fibrotic disease, namely pulmonary fibrosis. Studies in the mouse bleomycin-induced pulmonary fibrosis model suggest that exogenously administered ITAC can reduce collagen deposition, procollagen gene expression, histopathological fibroplasia, and extracellular matrix deposition in the lung.<sup>25</sup> In addition, ITAC reduced angiogenic activity and the total number of endothelial cells in the lung, suggesting that inhibition of pulmonary fibrosis is the result of inhibition of aberrant vascular remodeling. It is conceivable that a mechanism of action similar to that which inhibits neovascularization by suppressing angiogenesis<sup>26-28</sup> may account for the correlation between ITAC expression and improvement in liver fibrosis in response to IFN- $\gamma$ 1b therapy. Should this correlation be replicated in future studies, ITAC levels could potentially emerge as a useful predictor of response to IFN- $\gamma$ 1b antifibrotic therapy in chronic hepatitis C.

In conclusion, IFN- $\gamma$ 1b as a monotherapy for 48 weeks is not effective at reversing advanced fibrosis or cirrhosis. IFN- $\gamma$ 1b appears to have some antiviral effect in a minority of patients and seems to be well tolerated in most patients. The biomarker ITAC may be useful to determine if subgroups of patients with Ishak scores of <5-6 may benefit from IFN- $\gamma$ 1b alone or in combination with IFN- $\alpha$ . The study did not explore the impact of longer durations of IFN- $\gamma$ 1b therapy, with or without concomitant HCV antiviral therapy, on liver fibrosis or the impact of IFN- $\gamma$ 1b therapy on early-stage fibrosis.

**Table 6. Patients Who Died on Study (MITT Population, N = 488)**

Patient	Treatment*	Relatedness	Age	Primary Cause of Death
1	IFN-100	Possibly related	49	Decompensated cirrhosis
2	IFN-100	Unrelated	67	Unknown
3	IFN-100	Unrelated	41	Myocardial infarction
4	IFN-100	Unrelated	51	GI bleed
5	IFN-100	Possibly related	52	Multi-organ failure
6	IFN-200	Possibly related	53	Accidental overdose of heroin
7	IFN-200	Unrelated	68	Multisystem organ failure
8	IFN-200	Unrelated	51	Intractable GI bleed
9	IFN-200	Unrelated	61	Hepatorenal syndrome
10	IFN-200	Unrelated	46	Upper GI bleeding
11	IFN-200	Possibly related	50	Suicide
12	Placebo	Unrelated	50	Unknown
13	Placebo	Unrelated	58	Alberosclerotic cardiovascular disease
14	Placebo	Unrelated	56	Hepatocellular carcinoma
15	Placebo	Unrelated	55	Multisystem failure

\*IFN-100 = 100  $\mu$ g IFN- $\gamma$  1b; IFN-200 = 200  $\mu$ g IFN- $\gamma$  1b.

**Table 7. Clinical Manifestations of Decompensation (MITT Population, N = 488)**

	IFN- $\gamma$ 1b		Placebo (N = 162) n (%)
	200 $\mu$ g (N = 157) n (%)	100 $\mu$ g (N = 169) n (%)	
Number of patients with clinical manifestations of decompensation	21 (13%)	16 (9%)	15 (9%)
Type of clinical manifestations of decompensation			
Ascites	11 (7%)	8 (5%)	7 (4%)
GI bleeding due to varices	5 (3%)	2 (1%)	1 (1%)
Spontaneous bacterial peritonitis	0	1 (1%)	1 (1%)
Hepatic encephalopathy	6 (5%)	10 (6%)	6 (4%)
Hepatocellular carcinoma	3 (2%)	2 (1%)	3 (2%)
Death due to liver cirrhosis	1 (1%)	3 (2%)	1 (1%)

NOTE. Seven patients did not have hepatic decompensation assessments. Percentages are based on the number of patients in that treatment group.

## References

- Friedman SL. Evaluation of fibrosis and hepatitis C. *Am J Med* 1999; 107(Suppl):27S-30S.
- Nakamura T, Sakata R, Ueno T, Sata M, Ueno H. Inhibition of transforming growth factor beta prevents progression of liver fibrosis and enhances hepatocyte regeneration in dimethylnitrosamine-treated rats. *HEPATOLOGY* 2000;32:247-255.
- Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. *N Engl J Med* 1994;331:1286-1292.
- Ulloa L, Doody J, Massague J. Inhibition of transforming growth factor-beta/SMAD signalling by the interferon-gamma/STAT pathway. *Nature* 1999;397:710-713.
- Bolacchi F, Sinistro A, Ciaprini C, Demin F, Capozzi M, Carducci FC, et al. Increased hepatitis C virus (HCV)-specific CD4+CD25+ regulatory T lymphocytes and reduced HCV-specific CD4+ T cell response in HCV-infected patients with normal versus abnormal alanine aminotransferase levels. *Clin Exp Immunol* 2006;144:188-196.
- Ziesche R, Hofbauer E, Wittmann K, Petkov V, Block LH. A preliminary study of long-term treatment with interferon gamma-1b and low-dose prednisolone in patients with idiopathic pulmonary fibrosis. *N Engl J Med* 1999;341:1264-1269.
- Saez-Royuela F, Porres JC, Moreno A, Castillo I, Martinez G, Galiana F, et al. High doses of recombinant alpha-interferon or gamma-interferon for chronic hepatitis C: a randomized, controlled trial. *HEPATOLOGY* 1991;13:327-331.
- Soza A, Heller T, Ghany M, Lutchman G, Jake Liang T, Germain J, et al. Pilot study of interferon gamma for chronic hepatitis C. *J Hepatol* 2005; 43:67-71.
- Weng HL, Wang BE, Jia JD, Wu WF, Xian JZ, Mertens PR, et al. Effect of interferon gamma on hepatic fibrosis in chronic hepatitis B virus infection: a randomized controlled study. *Clin Gastroenterol Hepatol* 2005;3:819-828.
- Shiffman ML, Di Bisceglie AM, Lindsay KL, Morishima C, Wright EC, Everson GT, et al. Peg-interferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. *Gastroenterology* 2004;126:1015-1023.
- Poynard T, Schiff ER, Terg R, et al. Sustained virologic response (SVR) in the EPIC3 trial: week 12 virology predicts SVR in previous interferon/ribavirin treatment failures receiving PEG-Intron/Rebetol (PR) weight based dosing (WBD). *J Hepatol* 2005;42(Suppl 2):40-41.
- Curry M, Cardenas A, Afdhal NH. Effect of maintenance PEG-Intron therapy on portal hypertension and its complications: results from the COPILOT study. *J Hepatol* 2005;42(Suppl 2):40.
- Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncalves FL Jr, et al. Combination of peg-interferon alfa-2a plus ribavirin in patients with chronic hepatitis C virus infection. *N Engl J Med* 2002;347:975-982.
- Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004;140:346-355.
- Manns MP, McHutchison JG, Gordon S, Rustgi VK, Shiffman M, Rein-dollar R, et al. Peg-interferon alfa-2b plus ribavirin compared with inter-feron alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001;358:958-965.
- Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *HEPATOLOGY* 2003;38:1449-1457.
- Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *HEPATOLOGY* 2001;33:1358-1364.
- Patton HM, Patel K, Behling C, Bylund D, Blatt LM, Vallee M, et al. The impact of steatosis on disease progression and early and sustained virologic response in chronic hepatitis C patients. *J Hepatol* 2004;40:484-490.
- Poynard T, Ratziu V, McHutchison J, Manns M, Goodman Z, Zeuzem S, et al. Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. *HEPATOLOGY* 2003;38:75-85.
- Harrison SA, Brunt EM, Qazi RA, Oliver DA, Neuschwander-Tetri BA, Di Bisceglie AM, et al. Effect of significant steatosis or steatohepatitis on response to antiviral therapy in patients with chronic hepatitis C. *Clin Gastroenterol Hepatol* 2005;3:604-609.
- Fattovich G, Pantalena M, Zagni I, Realdi G, Schalm SW, Christensen E; European Concerted Action on Viral Hepatitis (EUROHEP). Effect of hepatitis B and C virus infections on the natural history of compensated cirrhosis: a cohort study of 297 patients. *Am J Gastroenterol*. 2002;97:2886-2895.
- Tan H, Derrick J, Hong J, Sanda C, Grosse WM, Edenberg HJ, et al. Global transcriptional profiling demonstrates the combination of type I and type II interferon enhances antiviral and immune responses at clinically relevant doses. *J Interferon Cytokine Res* 2005;25:632-649.
- Larkin J, Jin L, Farmen M, Venable D, Huang Y, Tan SL, et al. Synergistic antiviral activity of human interferon combinations in the hepatitis C virus replicon system. *J Interferon Cytokine Res* 2003;23:247-257.
- Okuse C, Rinaudo JA, Farrar K, Wells F, Korba BE, et al. Enhancement of antiviral activity against hepatitis C virus in vitro by interferon combination therapy. *Antiviral Res* 2005;65:23-34.
- Burdick MD, Murray LA, Keane MP, Xue YY, Zisman DA, Belperio JA, et al. CXCL11 attenuates bleomycin-induced pulmonary fibrosis via inhibition of vascular remodelling. *Am J Respir Crit Care Med* 2005;171:261-268.
- Romagnani P, Annunziato F, Lasagni L, Lazzeri E, Beltrame C, Francalanci M, et al. Cell cycle-dependent expression of CXC chemokine receptor 3 by endothelial cells mediates angiostatic activity. *J Clin Invest* 2001;107:53-63.
- Lasagni L, Francalanci M, Annunziato F, Lazzeri E, Giannini S, Cosmi L, et al. An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as functional receptor for platelet factor 4. *J Exp Med* 2003; 197:1537-1549.
- Keane MP, Belperio JA, Arenberg DA, Burdick MD, Xu ZJ, Xue YY, et al. IFN-gamma-inducible protein-10 attenuates bleomycin-induced pulmonary fibrosis via inhibition of angiogenesis. *J Immunol* 1999;163:5686-5692.