JOURNAL OF VIRAL HEPATITIS
JVH

Journal of Viral Hepatitis, 2013, **20,** 113–121 doi:10.1111/j.1365-2893.2012.01637.x

Role of IL-28B and inosine triphosphatase polymorphisms in efficacy and safety of Peg-Interferon and ribavirin in chronic hepatitis C compensated cirrhosis with and without oesophageal varices

V. Di Marco,[1] V. Calvaruso,[1] S. Grimaudo,[1] D. Ferraro,[2] R. M. Pipitone,[1] R. Di Stefano[2] and A. Craxì[1] [1]*Sezione di Gastroenterologia & Epatologia, Dipartimento Biomedico di Medicina Interna e Specialistica, University of Palermo, Palermo, Italy; and* [2]*Servizio di Virologia, Dipartimento di Scienze per la Promozione della Salute, University of Palermo, Palermo, Italy*

Received March 2012; accepted for publication May 2012

SUMMARY. Genetic factors can influence the outcome of antiviral therapy in chronic hepatitis C (HCV). We evaluated the role of interleukin-28B single nucleotide polymorphisms (SNPs) and inosine triphosphatase (ITPA) gene variants in HCV cirrhosis treated with Peg-Interferon and ribavirin. A prospective cohort of 233 patients with compensated cirrhosis received 1–1.5 μg/kg/week of Peg-Interferon alpha-2b plus 1000–1200 mg/day of RBV for 48 weeks. A sustained virologic response (SVR) was achieved in 27% of patients. On multivariate logistic analysis, the absence of oesophageal varices (OR 3.64 CI 95% 1.27–10.44 $P = 0.016$), infection with genotype 2 or 3 (OR 4.06, CI 95% 1.08–15.26, $P = 0.038$), C/C alleles of rs12979860 SNP (OR 7.04, CI 95% 2.40–20.72, $P < 0.001$) and rapid virologic response (RVR) (OR 78.29, CI 95% 16.07–381.29, $P < 0.001$) were independently associated with SVR. Patients who experienced post-treatment relapse received lower total doses of Peg-Interferon (52.0 ± 15.8 μg/kg *vs* 65.7 ± 13.3 μg/kg, $P < 0.001$) and lower total dose of RBV (3829 ± 1210 mg *vs* 4709 ± 954 mg, $P < 0.001$) than patients who achieved an SVR. ITPA variants predictive of high ITPase activity were associated with reduction of haemoglobin ≥3 g/dL in the first 4 weeks ($P < 0.001$), and with reduction of haemoglobin <10 g/dL ($P = 0.03$) on treatment. In conclusion, combination therapy with Peg-Interferon and RBV in patients with HCV cirrhosis must be guided by virus genotype, severity of portal hypertension, favourable IL-28B polymorphisms and ITPA variants, and RVR on treatment.

Keywords: chronic hepatitis C, cirrhosis, IL-28B, inosine triphosphatase, sustained virologic response.

INTRODUCTION

The prevalence of hepatitis C virus (HCV) cirrhosis and the incidence of its complications have increased recently in several countries because of the long-term persistence of HCV infection [1,2]. Identification and treatment of patients with HCV cirrhosis can reduce the number of cases of decompensation and hepatocellular carcinoma (HCC) [3–6]. There are few data on the efficacy and safety of combination therapy with Peg-Interferon (Peg-IFN) and ribavirin (RBV), in cirrhosis with portal hypertension, because these patients have been excluded from randomized controlled trials. Host factors such as age, gender, staging of liver fibrosis and steatosis, HCV genotypes, baseline HCV RNA levels and rapid disappearance of serum HCV RNA can influence the rate of sustained virologic response (SVR) [7,8].

Genome-wide association studies have identified single nucleotide polymorphisms (SNPs) located in and near the interleukin-28B (IL-28B) locus, which encodes for IFN-λ3 and is associated with a higher rate of SVR [9–11]. Two genetic variants of rs1127354 and rs7270101 SNPs in the inosine triphosphatase (ITPA) gene, which encode a protein

Abbreviations: AFP alpha-1-fetoprotein levels; ALT, alanine-aminotransferase; EPO, erythropoietin; ETR, end-of-treatment virologic response; EVR, early virologic response; GGT, gamma-glutamyl transferase; HCC, hepatocellular carcinoma; HCV, chronic hepatitis C; ITP, hydrolyses inosine triphosphate; ITPA, inosine triphosphatase gene; ITT, intention-to-treat; LVR, late virologic response; OV, oesophageal varices; PCR, polymerase chain reaction; Peg-IFN, Peg-Interferon; RBV, Ribavirin; RVR, rapid virologic response; SNPs, single nucleotide polymorphisms; SVR, sustained virologic response.

Correspondence: Vito Di Marco, Sezione di Gastroenterologia & Epatologia, Dipartimento Biomedico di Medicina Interna e Specialistica, University of Palermo, Piazza delle Cliniche 2, 90127 Palermo, Italy. E-mail: vito.dimarco@unipa.it

that hydrolyses inosine triphosphate (ITP), have been found to be associated with anaemia in patients treated with Peg-IFN and RBV [12–14].

As patients with cirrhosis have a low SVR rate and frequently have adverse events [15], it may be relevant to identify factors associated with higher probability of viral clearance, and to assess a patient's likelihood of SVR.

We studied a large prospective cohort of patients with compensated HCV cirrhosis to assess the safety and the efficacy of Peg-IFN and RBV, and to evaluate the association between virus-, genetic- and disease-related factors and SVR and the incidence of adverse events.

PATIENTS AND METHODS

Patients selection

In this independent, investigator-driven, prospective cohort study, we included all consecutive anti-HCV positive patients with METAVIR stage 4 at liver biopsy and/or presence of oesophageal varices (OV) observed at our Liver Unit between June 2003 and June 2007. Other inclusion criteria were age below 70 years, ultrasound scan (US) negative for focal lesions, values of alpha-1-fetoprotein levels (AFP) ≤20 ng/dL, bilirubin ≤2 mg/dL, albumin ≥2.8 g/dL, prothrombin time ≥60%, alanine-aminotransferase (ALT) upper normal limit, HCV RNA positive by polymerase chain reaction (PCR), naïve to antiviral treatment, or previously treated with interferon alone.

Exclusion criteria were previous decompensation of cirrhosis, HBsAg and/or anti-HIV positivity, history of alcohol abuse or IV drugs, platelet count $\leq 40 \times 10^3$/dL, leucocyte count $\leq 2.0 \times 10^3$/dL or absolute neutrophil count $\leq 1.0 \times 10^3$/dL, haemoglobin values ≤11 g/dL, creatinine ≥1.5 mg/dL, previous combination therapy with interferon or Peg-IFN plus RBV. The study protocol was approved by our hospital's Ethics Committee, and written consent was obtained from all patients.

Baseline evaluation

At baseline, a quantitative HCV RNA was performed by Cobas Monitor HCV v 2.0 (Roche Diagnostics, Basel, Switzerland), and HCV genotyping by Innolipa (Innogenetics, Belgium). All patients underwent clinical assessment including haematologic control, liver function tests, renal function tests, AFP serum levels, abdominal US and upper gastro-intestinal endoscopy to evaluate the presence and degree of OV.

Genetic tests

Patients were genotyped for *rs12979860* SNP of the IL-28B locus and for *rs1127354* and *rs7270101* SNPs of ITPA variants using serum samples collected at baseline and frozen at −80 °C. DNA was purified using the QIAmp Circulating Nucleic Acid Kit (Qiagen, Mainz, Germany), and DNA samples were quantified using spectrophotometric determination. To genotype the *rs12979860* SNP, we used a custom assay, while commercial genotyping assays were available for the *rs1127354* SNP (cat. C_27465000_10) and the rs7270101 SNP (C_29168507_10). The ABI TaqMan allelic discrimination method commercialized by Applied Biosystems was used, and the genotyping call was made with SDS software v.1.3.0 (ABI Prism 7500, Foster City, CA, USA). In accordance with previous reports [12,13], the severity of the ITPase deficiency variable was defined according to the allele combination of *rs1127354* and *rs7270101* SNPs. The combination of two wild-type alleles (C/C for *rs1127354* SNP and A/A for *rs7270101*) was predictive of absence of ITPase deficiency, while the combination with at least the presence of a minor allele (A for *rs1127354* SNP and C for *rs7270101*) in heterozygosis or homozygosis form was predictive of mild, moderate or severe ITPase deficiency.

Treatment schedules

Patients included in the cohort between June 2003 and March 2005 received 1 μg per kilogram per body weight weekly of Peg-IFN α_2b (PEG-Intron; Schering Plough, Merck & Co. Inc., (formerly Shering-Plough) Whitehouse Station, NJ, USA.) plus 1000 mg/daily (patients with body weight <70 kg) or 1200 mg/day (patients with body weight ≥70 kg) of oral RBV (Rebetol; Schering Plough) for 48 weeks. Patients included after March 2005 received 1.5 μg per kilogram per body weight weekly of Peg-IFN α_2b plus oral RBV at the same doses for 48 weeks. Granulocyte colony-stimulating factor (G-CSF) or erythropoietin (EPO) was used after March 2005, when Italian medical regulations allowed these drugs to be used for cirrhotic patients on combination antiviral therapy.

Clinical and virologic evaluation of therapy

All patients underwent haematologic, liver and renal function tests every 4 weeks on therapy and every 24 weeks during post-therapy follow-up. Abdominal US control and AFP were performed every 6 months to assess the HCC screening and surveillance. All patients were observed for at least 24 months. The study ended at the end of December 2009.

The primary virologic outcome was SVR, defined as undetectable HCV RNA by sensitive qualitative PCR assay with a cut-off <50 IU/mL (Amplicor HCV v 2.0 Roche Diagnostics) 24 weeks after cessation of therapy. Virologic outcomes of therapy, in accordance with European Association of the Study of the Liver Clinical Practice Guidelines (8), were rapid virologic response (RVR), defined as undetectable HCV RNA by PCR at 4 weeks of therapy; early

virologic response (EVR), defined as undetectable HCV RNA by PCR at 12 weeks of therapy; late virologic response (LVR), defined as undetectable HCV RNA by PCR at 24 weeks of therapy; end-of-treatment virologic response (ETR), defined as undetectable HCV RNA by PCR at 48 weeks of therapy for patients who completed entire treatment schedule, or at the last clinical control in patients who were withdrawn from therapy because of adverse events. Treatment was withdrawn after 24 weeks if HCV RNA was still positive.

Assessment of safety

Safety was assessed by haematologic control and liver function tests, evaluation of subjective adverse events and evidence of liver- and nonliver-related clinical events every month during treatment. Treatment was lowered or discontinued if severe adverse events occurred. Before March 2005, RBV was lowered to 600 mg/day if haemoglobin levels were lower than 10 g/dL, and was discontinued if the values fell below 8.5 g/dL. The dose of Peg-IFN was decreased by 50% if the total leucocyte count was $<1.5 \times 10^3$/dL or if the absolute neutrophil count was $<1.0 \times 10^3$/dL. Peg-IFN was discontinued if the total leucocyte count dropped to $<1.0 \times 10^3$/dL or the absolute neutrophil count dropped to $<0.75 \times 10^3$/dL and/or if the platelet count fell below 25×10^3/dL. When the drugs were stopped, they were not reintroduced.

After March 2005, when Italian medical regulations allowed G-CSF and EPO for HCV cirrhotic patients on antiviral combination therapy, patients with haemoglobin levels lower than 10 g/dL were treated with alpha erythropoietin at a dose of 10 000 IU three times a week or 40 000 times a week. Patients with an absolute neutrophil count $<0.75 \times 10^3$/dL were treated with G-CSF at the dose of 30 MU/week. RBV was lowered to 600 mg/day if haemoglobin levels were still lower than 10 g/dL after 4 weeks of erythropoietin therapy, and was discontinued if the values fell below 8.5 g/dL on erythropoietin therapy. Antiviral therapy was also withdrawn if ALT values exceeded 10 times upper normal limit and/or bilirubin rose beyond 4 mg/dL or if liver decompensation or HCC appeared.

To evaluate the association between the ITPase deficiency variants and incidence of anaemia during combination therapy, we analysed the rate of patients who experienced a reduction of haemoglobin levels ≥3 g/dL in the first 4 weeks of therapy or over the full course of 48 weeks, and the rate of patients who experienced a decrease in haemoglobin to <10 g/dL.

Statistical analysis

All data were entered into a database and analysed using SPSS 13.0 for Windows software (SPSS Inc., Chicago, IL, USA) on an intention-to-treat (ITT) basis. Continuous variables were expressed as mean ± standard deviation (SD) and categorical variables as absolute and relative frequencies. The differences between continuous data were analysed by t-test, and corrected chi-square analysis was used for dichotomous or categorical variables. Fisher's exact test was used to examine the association between baseline features and SVR. Multiple logistic regression analysis was used to identify baseline variables (age, gender, previous treatment with interferon monotherapy, diagnosis of diabetes, presence of OV, haemoglobin values, leucocyte and platelet counts, AST/ALT, gamma-glutamyl transferase (GGT), bilirubin, albumin values, prothrombin time, viral genotypes, serum HCV RNA levels, genotypes of *rs12979860* SNP, variants of ITPA deficiency, Peg-Interferon doses and RBV doses) and on treatment variables (RVR) associated with SVR. Variables with a threshold value of $P = 0.05$ at univariate analysis were included in the model, and variables with a threshold value of $P = 0.05$ were considered significant in the final model. The results were expressed as odds ratio (OR) and their 95% confidence intervals (CI). Cumulative incidence curves of haemoglobin reduction ≥3 g/L in relation to ITPase deficiency variants were estimated with the Kaplan–Meier method, and the differences between groups were assessed using logrank tests.

RESULTS

Baseline clinical features

The baseline clinical features of the 233 patients included in the cohort are shown in Table 1. One hundred and four patients (44.6%) had OV, and 22 of them (9.4%) were on primary prophylaxis with beta-blockers at the start of antiviral therapy because they had F2 OV. Sixty-five patients (27.9%) had a previous diagnosis of diabetes, and 75 patients (32.2%) had hypertension. Forty-eight patients (20.6%) had values $<75 \times 10^3$. The majority of patients (85%) were infected with HCV 1b genotype, and 73.4% of patients were naïve to antiviral therapy. The prevalence of C/C genotype of *rs12979860* SNP was 29%, while the distribution of ITPA variants was the same as that described by Thompson *et al.* [14,15]. One hundred and fourteen patients received 1 μg/kg/week of Peg-IFN α_2b plus 1000/1200 mg/daily of RBV for 48 weeks, and 119 patients received 1.5 μg/kg/week of Peg-IFN α_2b plus RBV at the same doses for 48 weeks.

Virologic response

On ITT analysis, 44 patients (18.9%) achieved an RVR, 52 patients (22.3%) achieved an EVR and 19 patients (8.1%) an LVR, with a total number of 115 patients (49.3%) who achieved an ETR. After 24 weeks of observation from the end of therapy, 63 patients (27.0%) achieved an SVR, while 52 patients (22.3%) showed a virologic relapse.

Table 1 Clinical, virologic and genetic features of the 233 patients

Gender	
Male	149 (64%)
Female	84 (36%)
Mean age	
Male	57.3 ± 9.6
Female	60.1 ± 6.7
Body weight (kg, mean, SD)	
Male	78.0 ± 12.9
Female	68.2 ± 13.4
Concomitant diseases	
Diabetes	65 (27.9%)
Hypertension	75 (32.2%)
Oesophageal varices	
Absent	129 (55.4%)
Present	104 (44.6%)
Child-pugh class	
A5	183 (78.5%)
A6	46 (19.7%)
B7	4 (1.7%)
Antiviral therapy	
Naïve patients	171 (73.4%)
Previously treated with IFN-alone	62 (26.7%)
HCV genotypes	
1	199 (85.4%)
2 or 3	34 (14.6%)
Serum HCV RNA (Log_{10}, median, range)	6.2 (2.1–8.1)
rs12979860 SNP genotypes*	
C/C	60 (29.1%)
T/C or T/T	146 (70.9%)
Predicted ITPase deficiency*	
Absent	133 (64.5%)
Mild	41 (17.6%)
Moderate/severe	32 (13.7%)
ALT (UI/mL, mean ± SD, normal values <40)	140.2 ± 88.7
GGT (UI/mL, mean ± SD, normal values <35)	112.5 ± 64.6
Bilirubin (mg/mL, mean ± SD)	1.0 ± 0.5
Albumin (g/mL, mean ± SD)	4.0 ± 04
Prothrombin time (%, mean ± SD)	86.7 ± 15.1
Haemoglobin (g/L, mean ± SD)	14.5 ± 1.3
Leucocytes (1×10^3, mean ± SD)	5.7 ± 1.8
Platelets (10×10^3, mean ± SD)	120 ± 47.7

ALT, alpha-1-fetoprotein levels; GGT, gamma-glutamyl transferase. *Evaluated in 206 patients.

Logistic regression modelling for sustained virologic response

We analysed baseline and on-therapy variables that were associated with SVR. On univariate analysis, absence of OV ($P = 0.016$), higher albumin ($P = 0.042$) and ALT values ($P = 0.009$), lower GGT values ($P = 0.035$), infection by genotype 2 or 3 ($P < 0.001$), C/C genotypes of *rs12979860* SNP ($P < 0.001$) and RVR ($P < 0.001$) were associated with SVR (Table 2). On multivariate logistic analysis, absence of OV (OR 3.64 CI 95% 1.27–10.44 $P = 0.016$), viral genotype 2 or 3 (OR 4.06, CI 95% 1.08–15.26, $P = 0.038$), C/C genotype of *rs12979860* SNP (OR 7.04, CI 95% 2.40–20.72, $P < 0.001$) and RVR (OR 78.29, CI 95% 16.07–381.29, $P < 0.001$) were independently associated with SVR.

Viral genotypes and virologic response

Only 25 (12.5%) of 199 genotype, 1 patient achieved an RVR *vs* 19 (55.9%) of 34 patients with genotype 2 or 3 ($P < 0.001$; Fig. 1). Rates of EVR and LVR were comparable for different genotypes (22.1% and 8.5% for genotype 1 and 23.5% and 5.9% for genotype 2 or 3, respectively). ETR was achieved in 86 patients (43.2%) with genotype 1, and in 29 patients (85.3%) with genotype 2 or 3 ($P < 0.001$). After the end of therapy, we observed a virologic relapse in 46 of 86 genotype 1 patients (53.5%) with ETR, but in only 6 of 29 genotype 2 or 3 patients (20.1%). Overall, 40 of 199 genotype 1 patients (20.1%) and 23 of 34 patients (67.6%) with genotype 2 or 3 achieved an SVR ($P < 0.001$).

Oesophageal varices and virologic response

Patients with and without OV achieved a comparable rate of virologic response on therapy. Patients with OV had a higher rate of relapse and, finally, the rate of SVR was significantly higher in patients without OV than in patients with OV (33.3% *vs* 19.2%, $P = 0.01$).

Time of virologic response on therapy and sustained virologic response

An RVR occurred in 25 of 199 genotype 1 patients, and in 19 of 34 genotype 2 or 3 patients (12.5% *vs* 55.9%, $P < 0.001$), while an EVR occurred in 44 of 199 genotype 1 patients, and in 8 of 34 genotype 2 or 3 patients (22.1% *vs* 23.5%, $P = 0.6$; Fig. 2). The rate of SVR was very high regardless of genotype in patients who achieved an RVR (92% in 25 genotype 1 patients and 84.2% in 19 genotype 2 or 3 patients), while it was correlated with genotype in patients who achieved an EVR (34.1% in 44 genotype 1 patients and 87.5% in 8 genotype 2 or 3 patients, $P = 0.007$).

IL-28B polymorphisms and virologic response

Polymorphism in the interleukin-28B was available in 206 patients (88.4%; Fig. 3). In the entire cohort, the C/C genotype of *rs12979860* SNP was significantly associated with the rate of SVR (51.6% in C/C patients *vs* 19.2% in C/T or T/T patients, $P < 0$ .001). The *rs12979860* SNP was not a determinant of SVR in genotype 1 patients who achieved

Table 2 Baseline and on-therapy variables associated with SVR by univariate and multivariate analysis

	No SVR (170 patients)	SVR (63 patients)	*P*	OR (CI 95%)	*P*
Age (SD)	58.9 ± 8.5	56.8 ± 9.5	0.100	–	
Gender (% males)	63	66	0.599	–	
Body weight (kg/m^2, SD)					
Male	77.7 ± 13.5	79.1 ± 11.9	0.888	–	
Female	69.3 ± 12.8	64.7 ± 14.7	0.085	–	
Oesophageal varices (%)					
Absent	50.5	68.3	**0.016**	3.64 (1.27–10.44)	**0.016**
Present	49.5	31.7			
Previous IFN therapy					
Yes	25.3	30.1	0.455	–	
No	74.7	69.9			
Haemoglobin (g/dL)	14.5 ± 1.4	14.5 ± 1.2	0.965	–	
Leucocytes (1×10^3)	5.6 ± 1.8	5.9 ± 1.8	0.266	–	
Platelets (10×10^3)	118 ± 46	127 ± 48	0.104	–	
Albumin (g/dL)	4.0 ± 0.4	4.1 ± 0.5	**0.042**	1.04 (0.42–2.42)	0.435
Bilirubin (mg/dL)	1.0 ± 0.6	0.9 ± 0.3	0.106	–	
AP (%)	86 ± 16	89 ± 14	0.234	–	
ALT (IU/mL)	132 ± 72	172 ± 108	**0.009**	1.05 (0.82–1.35)	0.687
GGT (IU/mL)	84 ± 59	66 ± 49	**0.035**	0.85 (0.60–1.21)	0.379
Genotype (%)					
1	93.5	63.5	**<0.001**	3.43 (0.94–12.56)	**0.061**
2/3	6.5	36.5			
HCV RNA (Log_{10})	6.01	6.21	0.232	–	
Diabetes (%)	28.8	25.4	0.604	–	
IL-28B (%)*					
C/C	19.7	52.5	<0.001	5.71 (2.08–15.70)	**0.001**
C/T or T/T	80.3	47.5			
ITPase deficiency variants (%)*					
No deficiency	65.3	62.6	0.748		
Mild deficiency	20.4	18.7			
Moderate/severe deficiency	14.3	18.7			
RVR (%)					
Yes	3	62	**<0.001**	62.87 (14.16–279.08)	**<0.001**
No	97	38			

ALT, alpha-1-fetoprotein levels; GGT, gamma-glutamyl transferase; RVR, rapid virologic response; SVR, sustained virologic response. *Evaluated in 206 patients. p values results ≤ 0.05 by multivariate analysis are signed in bold.

RVR (100% in 14 patients with C/C genotype and 87.5% in 8 patients with C/T or T/T genotype) but was a determinant in patients who achieved EVR (59% in 17 patients with C/C genotype and 19.2% in 26 patients with C/T or T/T genotype, $P = 0.01$).

Drugs doses and sustained virologic response

We evaluated Peg-IFN and RBV doses received by 115 patients who achieved an ETR. Higher total doses of Peg-IFN and RBV reduced the rate of post-treatment relapse. In fact, patients who experienced post-treatment relapse received lower total doses of Peg-IFN (52.0 ± 15.8 μg/kg *vs* 65.7 ± 13.3 μg/kg, $P < 0.001$) and lower total dose of RBV (3829 ± 1210 mg *vs* 4709 ± 954 mg, $P < 0.001$) than patients who achieved an SVR. When we evaluated only genotype 1 patients with ETR, the total dose of Peg-IFN (52.0 ± 15.8 μg/kg *vs* 65.7 ± 13.3 μg/kg, $P < 0.001$) and RBV (3829 ± 1210 mg *vs* 4709 ± 954 mg, $P < 0.001$) remained significantly lower in patients who experienced relapse than in patients who achieved an SVR.

Adverse events on therapy

Fifty-six patients (24%) were withdrawn from treatment because of adverse events (Table S1). Twenty-one patients

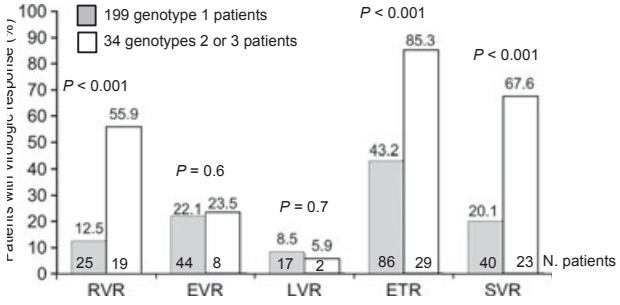

Fig. 1 Virologic response according to viral genotypes RVR, rapid virologic response; EVR, early virologic response; LVR, late virologic response; ETR, end-of-treatment virologic response; SVR, sustained virologic response.

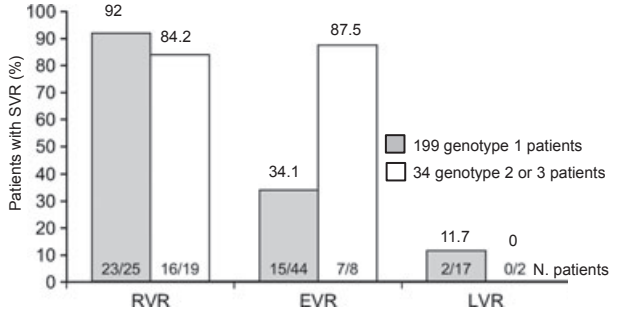

Fig. 2 Rates of sustained virologic response (SVR) according to time of virologic response on treatment, and viral genotypes RVR, rapid virologic response; EVR, early virologic response; LVR, late virologic response.

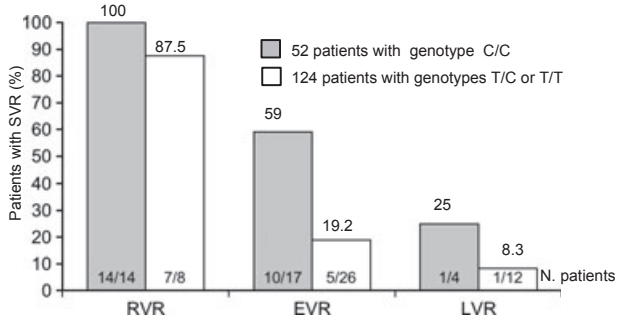

Fig. 3 Sustained virologic response (SVR) according to time of response on therapy and IL-28B single nucleotide polymorphisms in genotype 1 patients RVR, rapid virologic response; EVR, early virologic response; LVR, late virologic response.

(9.1%) stopped treatment because of specific events, with the most common event being severe fatigue. Eight patients (3.5%) discontinued treatment because of psychiatric or neurologic disorders, two patients (0.8%) because of vascular disorders and three patients (1.3%) contracted serious infection. Eight patients (3.4%) discontinued treatment because of liver-related complications: four developed ascites, on developed encephalopathy and one portal thrombosis. All of them had OV. One patient showed HCC at US control after 6 months of therapy, and another patient developed an ALT flare after 4 months of therapy. The laboratory abnormalities that caused treatment discontinuation were severe anaemia in seven patients (3%) and severe leukopenia in five patients (2.2%), while only two patients (0.8%) had severe thrombocytopenia.

Variants in the inosine triphosphatase gene and haemolytic anaemia

On treatment, 105 of 133 patients (78.9%) who carried variants predictive of high ITPase activity, 24 of 41 patients (58%) who carried polymorphisms associated with mild reduction in ITPase activity, and 16 of 32 patients (50%) who carried polymorphisms associated with moderate or severe reduction in ITPase activity experienced a reduction of haemoglobin levels ≥3 g/dL ($P < 0.001$ by logrank test; Fig. 4). Thirty of 133 of patients (22.5%) with high ITPase activity, but none of 41 patients with mild reduction in ITPase activity, and only one of 32 patients (3.1%) with a moderate or severe reduction in ITPase activity experienced a reduction of haemoglobin levels ≥3 g/dL in the first 4 weeks of treatment ($P < 0.001$). Finally, patients with high ITPase activity had a significantly higher risk of reduction of haemoglobin values to <10 g/dL than patients with mild, moderate or severe reduction in ITPase activity (51/133 *vs* 17/73, $P = 0.03$).

Among 71 patients who experienced a reduction of haemoglobin values to <10 g/dL, 44 patients used EPO as support therapy and 27 adjusted RBV doses. Eighteen of 44 patients (41%) who used EPO achieved an SVR, while five of 27 patients (18.5%) who reduced RBV doses achieved an SVR ($P = 0.06$).

DISCUSSION

The efficacy of antiviral therapy with Peg-IFN and RBV is evidence based in patients with cirrhosis, as the eradication of HCV significantly reduces the incidence of disease complications and increases survival [4–6]. In this setting of patients, the overall rate of SVR is <30%, with a clear difference between genotype 1 or 4 patients who achieved an SVR in <20% of cases, and genotype 2 or 3 patients who achieved an SVR in more than 50% of cases [16–18]. In addition, the rate of therapy withdrawal because of adverse events was higher in patients with cirrhosis than in those with chronic hepatitis [15].

This is the first study to evaluate viral factors, genetic variants, disease features, drugs doses and kinetics of virologic responses that are associated with antiviral therapy outcomes in patients with advanced, but not decompensated, cirrhosis.

About half of the patients included in our cohort had OV and 20% had platelet counts $<75 \times 10^3$. As reported by the

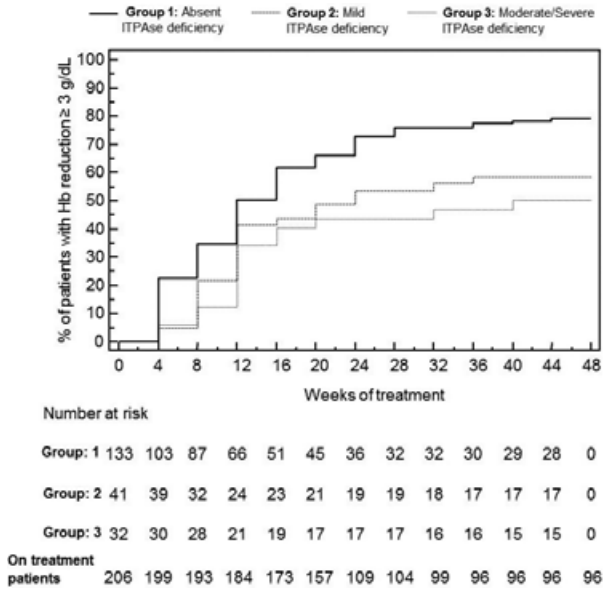

Fig. 4 Incidence of haemoglobin reduction ≥3 g/L during treatment according to ITPase deficiency variants identified by Kaplan–Meier method ($P < 0.001$ by logrank test).

studies of the HALT-C Trial Group, patients with these characteristics have a low probability of achieving an SVR [19] and a high probability of developing or increasing OV [20] and of developing HCC [21] during the follow-up. In this setting of 'difficult to treat patients', it is crucial to identify predictors of SVR and host factors associated with the development of severe adverse events on therapy and to understand the kinetics of virologic response to apply a response-guided strategy [8].

The overall rate of SVR in our study was 27%, with no significant differences among the patients in terms of age, gender and BMI. SVR was similar in naïve patients and patients previously treated with interferon alone. By contrast, absence of OV, 'easy' viral genotypes, 'favourable' IL-28B polymorphisms and RVR on therapy were all independently associated with SVR. Patients with OV achieved the same rate of virologic response on therapy as patients without OV, but they had a higher rate of relapse, and therefore, the rate of SVR was significantly higher in patients without OV. It is difficult to explain the mechanisms underlying these data, but it is likely that the progression of fibrosis simultaneously increases the portal pressure and reduces the possibility of HCV clearance [22].

The rate of SVR was 68% in genotype 2 patients, and 66% in genotype 3 patients. In this subset of patients, the success of therapy was not related to IL-28B polymorphisms and not conditioned by RVR. It is unclear whether these patients require 48 weeks of therapy or could be treated with a cycle of 24 weeks. A recent study [18] suggests that treatment failure was the consequence of higher rates of post-treatment relapse in genotype 2 or 3 cirrhotic patients than in patients with chronic hepatitis treated for 24 weeks and reported a rate of 29% and 61% in genotype 2 and genotype 3 cirrhotic patients, respectively. In our study, all patients were treated for 48 weeks, and the rate of post-treatment relapse was 19% in genotype 2 patients, and 25% in genotype 3 patients. These data reinforce the indication for PEG-IFN and RBV in genotype 2 and 3 patients with compensated cirrhosis and confirm the suggestion that this group of patients may benefit from extended duration of treatment [23].

Only 20.1% of genotype 1 patients achieved an SVR, and this low rate of success was related to IL-28B polymorphisms and the time of virologic response during treatment. First, the low rate of SVR may be related to low prevalence of C/C genotype of *rs12979860* SNP among genotype 1 cirrhotics, and to the low rate of RVR. Second, we observed a high post-treatment relapse rate in patients who carried a C/T or TT of *rs12979860* SNP and who achieved EVR or LVR. This suggests that genotype 1 patients who carry C/C genotypes of *rs12979860* SNP and achieve an RVR have a very high likelihood of achieving an SVR with 48 weeks of combination therapy, but patients with C/T or TT genotypes of *rs12979860* SNP who do not achieve an RVR have a very low likelihood of obtaining clearance of HCV and should be considered for new therapies with direct-acting antivirals [24,25].

Finally, we observed that the rate of SVR in the 115 patients who achieved an ETR was related to higher doses of Peg-IFN and RBV. Analysis of the SVR rate in patients with a virologic response showed that patients who experienced post-treatment relapse received lower doses of Peg-IFN and Ribavirin than patients who achieved an SVR. This observation strengthens the hypothesis that the intensification of treatment is a possible approach to reducing the incidence of relapse [26].

Regarding the safety of therapy, we observed that types and frequencies of adverse events were different from those reported in a larger randomized trial [26]. Twenty-four per cent of our patients stopped therapy because of severe adverse events, and we observed some events that were related to cirrhosis. All patients who experienced serious infection and liver decompensation had OV, a low platelet count and increased bilirubin levels. As these clinical features predict liver decompensation [27], and infections in patients with cirrhosis increase mortality [28], the choice of antiviral therapy in these patients must be carefully evaluated and weighed. The rate of patients whose haemoglobin values had reduced to <10 g/dL was similar to that reported in a previous study [26], and the use of EPO seems to influence the efficacy of therapy because it allows maintenance of the standard regimen of RBV.

In conclusion, our study confirms that combination therapy with Peg-Interferon and RBV is effective and safe in patients with cirrhosis and portal hypertension, but that before applying it, one must consider the clinical and genetic characteristics of patients, the viral genotypes and the kinetics of viral response. In genotype 2 or 3 patients, the administration of combination therapy for 48 weeks can

achieve results similar to those obtained in patients with chronic hepatitis. In genotype 1 patients, the therapy must be guided by the presence of OV, genetic variants and by RVR. It is also worth noting that patients with severe portal hypertension may have an increased risk of developing complications related to liver disease during treatment.

CONFLICT OF INTEREST

V. Di Marco has been invited speaker for Schering Plough, Roche, Gilead, BMS. A. Craxì has been a consultant and invited speaker for Roche, Schering Plough, Gilead, BMS and Novartis. Other authors have no financial disclosure to declare.

REFERENCES

1 Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol* 2006; 45: 529–538.
2 Davis GL, Albright JE, Cook SF, Rosenberg DM. Projecting future complications of chronic hepatitis C in the United States. *Liver Transpl* 2003; 9: 331–338.
3 Coverdale SA, Khan MH, Byth K *et al.* Effects of Interferon treatment response on liver complications of chronic hepatitis C: 9-year follow up study. *Am J Gastroenterol* 2004; 99: 636–644.
4 Shiratori Y, Ito Y, Yokosuka O, Imazeki F, Nakata R, Tanaka N. Antiviral therapy for cirrhotic hepatitis C: association with reduced hepatocellular carcinoma development and improved survival. *Ann Intern Med* 2005; 142: 105–114.
5 Bruno S, Stroffolini T, Colombo M *et al.* Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 2007; 45: 579–587.
6 Veldt BJ, Heathcote EJ, Wedemeyer H *et al.* Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. *Ann Intern Med* 2007; 147: 677–684.
7 Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; 49: 1335–1374.
8 Craxì A. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *Liver International* 2012; 32, Suppl. 1: 2–8.
9 Ge D, Fellay J, Thompson AJ *et al.* Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; 461: 399–401.
10 Tanaka Y, Nishida N, Sugiyama M *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and Ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; 41: 1105–1109.
11 Suppiah V, Moldovan M, Ahlenstiel G *et al.* IL28B is associated with response to chronic hepatitis C interferon-alpha and Ribavirin therapy. *Nat Genet* 2009; 41: 1100–1104.
12 Fellay J, Thompson AJ, Ge D *et al.* ITPA gene variants protect against anemia in patients treated for chronic hepatitis C. *Nature* 2010; 464: 405–408.
13 Thompson AJ, Fellay J, Patel K *et al.* Variants in the ITPA gene protect against Ribavirin-induced hemolytic anemia and decrease the need for Ribavirin dose reduction. *Gastroenterology* 2010; 139: 1181–1189.
14 Thompson AJ, Santoro R, Piazzolla V *et al.* Inosine triphosphatase genetic variants are protective against anemia during antiviral therapy for HCV2/3 but do not decrease dose reductions of RBV or increase SVR. *Hepatology* 2011; 53: 389–395.
15 Vezali E, Aghemo A, Colombo M. A review of the treatment of chronic hepatitis C virus infection in cirrhosis. *Clin Ther* 2010; 32: 2117–2138.
16 Di Marco V, Almasio PL, Ferraro D *et al.* Peg-interferon alone or combined with Ribavirin in HCV cirrhosis with portal hypertension: a randomized controlled trial. *J Hepatol* 2007; 47: 484–491.
17 Giannini EG, Basso M, Savarino V, Picciotto A. Predictive value of on treatment response during full-dose antiviral therapy of patients with hepatitis C virus cirrhosis and portal hypertension. *J Intern Med* 2009; 266: 537–546.
18 Aghemo A, Rumi MG, Monico S *et al.* The pattern of pegylated interferon-alpha2b and Ribavirin treatment failure in cirrhotic patients depends on hepatitis C virus genotype. *Antivir Ther* 2009; 14: 577–584.
19 Everson GT, Hoefs JC, Seeff LB *et al.*, for HALT-C Trial Group. Impact of disease severity on outcome of antiviral therapy for chronic hepatitis C: lessons from the HALT-C trial. *Hepatology* 2006; 44: 1675–1684.
20 Fontana RJ, Sanyal AJ, Ghany MG *et al.*, for HALT-C Trial Group. Factors that determine the development and progression of gastroesophageal varices in patients with chronic hepatitis C. *Gastroenterology* 2010; 138: 2321–2331.
21 Lok AS, Seeff LB, Morgan TR *et al.*, for HALT-C Trial Group. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* 2009; 136: 138–148.
22 Calvaruso V, Di Marco V, Bronte F, Cabibi D, Bavetta MG, Craxì A. Measurement of collagen proporzionate area in HCV cirrhosis has major clinical correlates. *Hepatology* 2010; 52(Suppl 4): 1582 A.
23 Zeuzem S, Berg T, Moeller B *et al.* Expert opinion on the treatment of patients with chronic hepatitis C. *J Viral Hepat* 2009; 16: 75–90.
24 Jacobson IM, McHutchison JG, Dusheiko G *et al.* Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; 364: 2405–2416.
25 Poordad F, McCone Jr J, Bacon BR *et al.* Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; 364: 1195–1206.
26 McHutchison JG, Lawitz EJ, Shiffman ML *et al.* IDEAL Study Team. Peg-Interferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *N Engl J Med* 2009; 361: 580–593.
27 D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic

indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; 44: 217–231.

28 Arvaniti V, D'Amico G, Fede G *et al.* Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology* 2010; 139: 1246–1256.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Table S1. Severe adverse events that caused discontinuation of treatment.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.