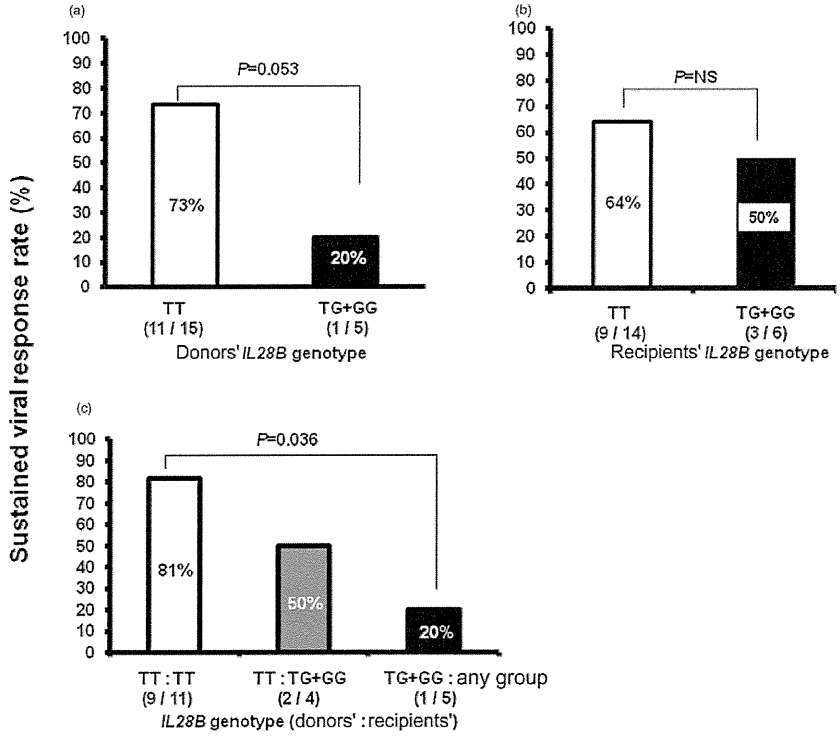

Figure 2 Sustained viral response rates according to (a) donors' interleukin-28B (*IL28B*), (b) recipients' *IL28B*, and (c) donors' and recipients' *IL28B* in patients infected with hepatitis C virus genotype 1. TT : TT group (donors' *IL28B* TT: recipients' *IL28B* TT), TT : TG + GG group (donors' *IL28B* TT: recipients' *IL28B* TG + GG), TG + GG : any group (donors' *IL28B* TG + GG: recipients' *IL28B* either TT or TG + GG). NS, not significant.

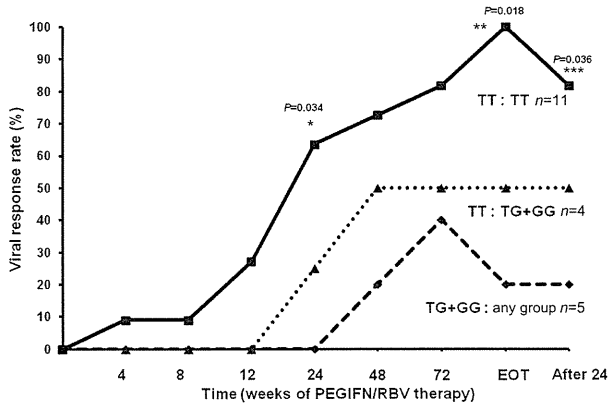

Figure 3 Viral response rates according to donors' and recipients' interleukin-28B (*IL28B*) genotyping. TT : TT group (donors' *IL28B* TT: recipients' *IL28B* TT), TT : TG + GG group (donors' *IL28B* TT: recipients' *IL28B* TG + GG), TG + GG : any group (donors' *IL28B* TG + GG: recipients' *IL28B* either TT or TG + GG). *Viral rate (VR) of the TT : TT group was 63.6% ($n = 7/11$), which was higher than the VR rate of the TG + GG : any group (0%, $n = 0/5$) at 24 weeks. **VR rate of the TT : TT group was 100% ($n = 11/11$), which was higher than the VR rate of the TG + GG : any group (20%, $n = 1/5$) at the end of treatment (EOT). ***Sustained VR (SVR) rate of the TT : TT group was 100% ($n = 11/11$), which was higher than the SVR rate of the TG + GG : any group (20%, $n = 1/5$) at 24 weeks at the EOT. PEGIFN, pegylated interferon; RBV, ribavirin.

Discussion

The SVR rate has improved since the introduction of PEGIFN/RBV for patients who undergo LT for HCV-related end-stage liver disease. The current estimated SVR rate for LT patients with a history of HCV-1 infection is 30–50%.[21–24,26,27] These results are much better than those reported in the 1990s and early 2000s; however, more than half of recipients still suffer from recurrent chronic hepatitis C.

Although many studies have determined the predictive factors of the viral response for PEGIFN/RBV among patients with chronic hepatitis C, recent molecular biological analyses and genome-wide analyses of the human genome have identified genetic variations of *IL28B* and amino-acid substitution of HCV core 70 as the most significant predictive factors for IFN response.[3–5,32,33] *IL28B* encodes a cytokine distantly related to type I IFN and the IL-10 family. It has been reported that the expression level of the *IL28* gene in peripheral blood mononuclear cells is significantly lower in individuals with minor alleles than in individuals with major alleles.[5]

Several studies have determined the predictive factors for the viral response to PEGIFN/RBV in patients with recurrent post-LT hepatitis C viral infection, and recent molecular and genome wide analyses of the human genome have demonstrated that genetic variation of *IL28B* is the most significant predictive factor of the response to IFN.[8,34–37] In the present study, we examined whether the same factors can also predict the response to PEGIFN/RBV in LT recipients. Several groups have reported that recipients' and donors' *IL28B* influenced the SVR to PEGIFN/RBV in patients with recurrent hepatitis C after LT.[8,36,37] Furthermore, others

Table 2 Univariate analysis of factors associated with sustained viralresponse (SVR) during interferon therapy in genotype 1 patients with recurrent hepatitis C

	SVR ($n = 12$)	Non-SVR ($n = 8$)	*P*-value
Age (years)†	60 (44–69)	57 (47–65)	0.48
Sex (male/female)	10/2	5/3	0.3
Body mass index (kg/m^2)†	24.1 (21.4–26.5)	24.2 (18.9–42.2)	0.4
Viral load at therapy (LogIU/mL)†	6.3 (5.8–6.6)	6.6 (5.9–7.2)	0.52
Time from transplantation to therapy (months)†	4 (1–41)	3 (1–6)	1.7
No. mutations in the ISDR (0–1/2–5)	7/5	5/3	1.0
HCV core70 region (mutant/wild)	7/5	5/3	1.0
HCV core 91 region (mutant/wild)	7/5	3/5	0.6
Donors' *IL28B* genotype TT/TG + GG	11/1	4/4	0.053
Recipients' *IL28B* genotype TT/TG + GG	9/3	5/3	0.6
Donors' and recipients' *IL28B* genotype TT : TT/others	9/3	2/6	0.037
Immunosuppression (tacrolimus/cyclosporine)	9/3	7/1	1.0
Adherence to PEGIFN ≥ 70/< 70 (%)†	8/4	3/5	0.3
Adherence to RBV ≥ 50/< 50 (%)†	7/5	1/7	0.076

†Values are median (range). HCV, hepatitis C virus; *IL28B*, interleukin-28B; ISDR, interferon sensitivity-determining region; PEGIFN, pegylated interferon; RBV, ribavirin.

reported that donors' *IL28B* influenced the SVR in patients treated with PEGIFN/RBV for recurrent hepatitis C after LT,[34] and that recipients' *IL28B* influenced the SVR to PEGIFN/RBV in patients with recurrent post-LT hepatitis C.[35,36]

The results of the present study indicate that both donors' and recipients' *IL28B* influence the SVR to PEGIFN/RBV in patients with recurrent post-LT hepatitis C. Both recipients' and donors' *IL28B* influenced the SVR to PEGIFN/RBV in recurrent hepatitis C after LT; however it is not clear whether the recipients' or donors' *IL28B* influenced the SVR to PEGIFN/RBV.

However, the donors' *IL28B* might have influenced the SVR to PEGIFN/RBV in patients with recurrent post-LT hepatitis C more than the recipients' *IL28B*. This conclusion is based on the following results: although the SVR rate of the TT group (64.2%) was similar to that of the TG + GG group (50%), according to the recipients' *IL28B*, the SVR rate of the TT group (73.3%) was higher than that of the TG + GG group (20%), according to the donors' *IL28B*. Furthermore, the VR rates of TT : TT, TT : TG + GG, TG + GG : any group at 12 weeks were 28%, 0%, and 0%; those at 48 weeks were 70%, 50%, and 20%; and those at the end of treatment were 100%, 50%, and 20%, respectively. That is, the time to VR of the TG + GG : any group was the latest among the three groups. Lange *et al.* reported that donors' *IL28B* influenced the SVR in patients treated with PEGIFN/RBV for recurrent hepatitis C after LT.[34] In this regard, Hiraga *et al.*[38] reported that IFN-stimulated gene expression levels in mice livers measured at 2 weeks after IFN treatment were significantly higher in mice transplanted with donor human hepatocytes (*IL28B*; TT) than from donor (*IL28B*; TG + GG) mice. Furthermore, previous studies reported that the expression level of IFN- λ-3, coded for the *IL28B* gene, was higher in hepatocytes than hematopoietic cells.[39]

However, we demonstrated the feasibility of treatment of LT recipients with PEGIFN/RBV until HCV–RNA reached undetectable levels, followed by the continuation of treatment for at least 48 weeks (i.e. long-term IFN therapy). In fact, the SVR rate (50%) of the recipients' *IL28B* TG + GG group was higher than that reported by others[8] (SVR rate: 11%). Furthermore, the SVR rate (81%) of the combination of donors' and recipients' *IL28B* (TT : TT) group was higher than that reported by Fukuhara *et al.*[8] (SVR rate: 56%). However, the SVR rate of the donors' *IL28B* TG + GG group (SVR rate: 20%) was similar to that reported by Fukuhara *et al.*[8] (SVR rate: 9%). We believe that the treatment of LT recipients with PEGIFN/RBV until HCV–RNA reaches undetectable levels, followed by the continuation of treatment for at least 48 weeks, is not useful for donors with *IL28B* TG + GG.

In Japan, LDLT is more common than orthotopic LT. In finding a suitable donor, it is better to select a donor with TT of the *IL28B* gene than a TG or GG donor. In conclusion, our results demonstrated the suitability of donors with the TT *IL28B* genotype, and that long-term PEGIFN/RBV therapy seems useful for recipients of LDLT who develop recurrent hepatitis C after transplantation.

References

1 Szabo E, Lotz G, Paska C, Kiss A, Schaff Z. Viral hepatitis: new data on hepatitis C infection. *Pathol. Oncol. Res.* 2003; **9**: 215–21.
2 Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; **39**: 1147–71.
3 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**: 1100–4.
4 Ge D, Fellay J, Thompson AJ *et al.* Genetic variation in *IL28B* predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399–401.
5 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**: 1105–9.
6 Thomas DL, Thio CL, Martin MP *et al.* Genetic variation in *IL28B* and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**: 798–801.
7 Rauch A, Kutalik Z, Descombes P *et al.* Genetic variation in *IL28B* is associated with chronic hepatitis C and treatment failure: a

genome-wide association study. *Gastroenterology* 2010; **138**: 1338–45, 45 e1–7.
8 Fukuhara T, Taketomi A, Motomura T *et al.* Variants in *IL28B* in liver recipients and donors correlate with response to peg-interferon and ribavirin therapy for recurrent hepatitis C. *Gastroenterology* 2010; **139**: 1577–85, 85 e1–3.
9 Sanchez-Fueyo A, Restrepo JC, Quinto L *et al.* Impact of the recurrence of hepatitis C virus infection after liver transplantation on the long-term viability of the graft. *Transplantation* 2002; **73**: 56–63.
10 Forman LM, Lewis JD, Berlin JA, Feldman HI, Lucey MR. The association between hepatitis C infection and survival after orthotopic liver transplantation. *Gastroenterology* 2002; **122**: 889–96.
11 Berenguer M, Prieto M, San Juan F *et al.* Contribution of donor age to the recent decrease in patient survival among HCV-infected liver transplant recipients. *Hepatology* 2002; **36**: 202–10.
12 Feray C, Caccamo L, Alexander GJ *et al.* European collaborative study on factors influencing outcome after liver transplantation for hepatitis C. European Concerted Action on Viral Hepatitis (EUROHEP) Group. *Gastroenterology* 1999; **117**: 619–25.
13 Gane E. The natural history and outcome of liver transplantation in hepatitis C virus-infected recipients. *Liver Transpl.* 2003; **9**: S28–34.
14 Prieto M, Berenguer M, Rayon JM *et al.* High incidence of allograft cirrhosis in hepatitis C virus genotype 1b infection following transplantation: relationship with rejection episodes. *Hepatology* 1999; **29**: 250–6.
15 Velidedeoglu E, Mange KC, Frank A *et al.* Factors differentially correlated with the outcome of liver transplantation in HCV+ and HCV– recipients. *Transplantation* 2004; **77**: 1834–42.
16 Samuel D, Feray C. Recurrent hepatitis C after liver transplantation: clinical and therapeutical issues. *J. Viral Hepat.* 2000; **7**: 87–92.
17 Thomas RM, Brems JJ, Guzman-Hartman G, Yong S, Cavaliere P, Van Thiel DH. Infection with chronic hepatitis C virus and liver transplantation: a role for interferon therapy before transplantation. *Liver Transpl.* 2003; **9**: 905–15.
18 Ueda Y, Takada Y, Haga H *et al.* Limited benefit of biochemical response to combination therapy for patients with recurrent hepatitis C after living-donor liver transplantation. *Transplantation* 2008; **85**: 855–62.
19 Sugawara Y, Makuuchi M, Matsui Y *et al.* Preemptive therapy for hepatitis C virus after living-donor liver transplantation. *Transplantation* 2004; **78**: 1308–11.
20 Sugawara Y, Makuuchi M. Living donor liver transplantation to patients with hepatitis C virus cirrhosis. *World J. Gastroenterol.* 2006; **12**: 4461–5.
21 Roche B, Sebagh M, Canfora ML *et al.* Hepatitis C virus therapy in liver transplant recipients: response predictors, effect on fibrosis progression, and importance of the initial stage of fibrosis. *Liver Transpl.* 2008; **14**: 1766–77.
22 Lodato F, Berardi S, Gramenzi A *et al.* Clinical trial: peg-interferon alfa-2b and ribavirin for the treatment of genotype-1 hepatitis C recurrence after liver transplantation. *Aliment. Pharmacol. Ther.* 2008; **28**: 450–7.
23 Dinges S, Morard I, Heim M *et al.* Pegylated interferon-alpha2a/ribavirin treatment of recurrent hepatitis C after liver transplantation. *Transpl. Infect. Dis.* 2009; **11**: 33–9.
24 Saab S, Oh MK, Ibrahim AB *et al.* Anemia in liver transplant recipients undergoing antiviral treatment for recurrent hepatitis C. *Liver Transpl.* 2007; **13**: 1032–8.
25 Kawaoka T, Hiraga N, Takahashi S *et al.* Prolongation of interferon therapy for recurrent hepatitis C after living donor liver transplantation: analysis of predictive factors of sustained virological response, including amino acid sequence of the core and NS5A regions of hepatitis C virus. *Scand. J. Gastroenterol.* 2010; **45**: 1488–96.
26 Tamura S, Sugawara Y, Yamashiki N, Kaneko J, Kokudo N, Makuuchi M. Pre-emptive antiviral therapy in living donor liver transplantation for hepatitis C: observation based on a single-center experience. *Transpl. Int.* 2009; **6**: 580–588.
27 Ueda Y, Takada Y, Marusawa H, Egawa H, Uemoto S, Chiba T. Individualized extension of pegylated interferon plus ribavirin therapy for recurrent hepatitis C genotype 1b after living-donor liver transplantation. *Transplantation* 2010; **6**: 661–5.
28 Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y. A high-throughput SNP typing system for genome-wide association studies. *J. Hum. Genet.* 2001; **46**: 471–7.
29 Suzuki A, Yamada R, Chang X *et al.* Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat. Genet.* 2003; **34**: 395–402.
30 Kitamura S, Tsuge M, Hatakeyama T *et al.* Amino acid substitutions in core and NS5A regions of the HCV genome can predict virological decrease with pegylated interferon plus ribavirin therapy. *Antivir. Ther.* 2010; **15**: 1087–97.
31 Mori N, Imamura M, Kawakami Y *et al.* Randomized trial of high-dose interferon-alpha-2b combined with ribavirin in patients with chronic hepatitis C: correlation between amino acid substitutions in the core/NS5A region and virological response to interferon therapy. *J. Med. Virol.* 2009; **81**: 640–9.
32 Akuta N, Suzuki F, Kawamura Y *et al.* Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. *J. Med. Virol.* 2007; **79**: 1686–95.
33 Akuta N, Suzuki F, Kawamura Y *et al.* Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J. Hepatol.* 2007; **46**: 403–10.
34 Lange CM, Moradpour D, Doehring A *et al.* Impact of donor and recipient IL28B rs12979860 genotypes on hepatitis C virus liver graft reinfection. *J. Hepatol.* 2011; **55**: 322–7.
35 Eurich D, Boas-Knoop S, Ruehl M *et al.* Relationship between the interleukin-28b gene polymorphism and the histological severity of hepatitis C virus-induced graft inflammation and the response to antiviral therapy after liver transplantation. *Liver Transpl.* 2011; **17**: 289–98.
36 Coto-Llerena M, Perez-Del-Pulgar S, Crespo G *et al.* Donor and recipient *IL28B* polymorphisms in HCV-infected patients undergoing antiviral therapy before and after liver transplantation. *Am. J. Transplant.* 2011; **11**: 1051–7.
37 Charlton MR, Thompson A, Veldt BJ *et al.* Interleukin-28B polymorphisms are associated with histological recurrence and treatment response following liver transplantation in patients with hepatitis C virus infection. *Hepatology* 2011; **53**: 317–24.
38 Hiraga N, Abe H, Imamura M *et al.* Impact of viral amino acid substitutions and host interleukin-28b polymorphism on replication and susceptibility to interferon of hepatitis C virus. *Hepatology* 2011; **3**: 764–71.
39 Miller DM, Klucher KM, Freeman JA, Hausman DF, Fontana D, Williams DE. Interferon lambda as a potential new therapeutic for hepatitis C. *Ann. N. Y. Acad. Sci.* 2009; **1182**: 80–7.

Case Report

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Achievement of Sustained Viral Response after Switching Treatment from Pegylated Interferon α-2b to α-2a and Ribavirin in Patients with Recurrence of Hepatitis C Virus Genotype 1 Infection after Liver Transplantation: A Case Report

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Key Words
PEG-IFN α-2a · PEG-IFN α-2b · *IL28B* · Hepatitis C virus · Liver transplantation

Abstract
We report a case in which sustained viral response was achieved after switching treatment from pegylated interferon (PEG-IFN) α-2b to α-2a and ribavirin (RBV) in patients with recurrence of hepatitis C virus (HCV) infection after living donor liver transplantation. The patient was a 62-year-old man with liver cirrhosis due to HCV genotype 1b infection. The patient had 8 amino acid (aa) substitutions in the interferon sensitivity-determining region, and had substitutions for mutant and wild-type at aa70 and aa91, respectively, in the core region. The patient had minor genotype (GG) *IL28B* single nucleotide polymorphisms (rs8099917). He had initially received interferon α-2b and RBV for 2 years, and later developed hepatocellular carcinoma (HCC). After surgical resection of HCC, he subsequently received PEG-IFN α-2b and RBV for 1.5 years, without undetectable viremia during the treatment course. Due to recurrence of HCC, the patient received a living donor liver transplantation. Later on, hepatitis C relapsed. For the management of relapse, he received another course of PEG-IFN α-2b and RBV. However, breakthrough viremia occurred. PEG-IFN was thus switched from α-2b to α-2a and RBV for another 17 months. The patient eventually achieved a sustained viral response.

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Introduction

Currently, pegylated interferon (PEG-IFN) α and ribavirin (RBV) are used as standard therapy for the treatment of patients with hepatitis C virus (HCV) infection; successful outcomes with PEG-IFN and RBV have been achieved in approximately 60% of the treated cases [1]. However, about 50% of the patients treated with this therapy have been reported to show an increase in the viral load and/or serum alanine aminotransferase (ALT) level during therapy [2, 3]. The event of increase in the viral load is called 'breakthrough viremia'. No treatment regimens have been established for patients who develop breakthrough viremia during treatment with or relapse following PEG-IFN and RBV. In some reports, PEG-IFN α-2a and RBV has been reported to result in a higher sustained viral response (SVR) than that achieved by PEG-IFN α-2b and RBV [4]. Moreover, PEG-IFN α-2a and RBV was reported to be effective for treatment of some HCV patients who experienced relapse after PEG-IFN α-2b and RBV [5]. Although there are reports about interferon (IFN) therapy in patients with HCV genotype 1 infection after liver transplantation (LT) [6–8], there are no reported cases where SVR was achieved by switching treatment with PEG-IFN from α-2b to α-2a and RBV in patients with HCV genotype 1 infection after LT.

We report a case in whom SVR was achieved after switching treatment from PEG-IFN α-2b to α-2a and RBV in a patient with recurrence of HCV genotype 1 infection after LT.

Case Report

The patient was a 62-year-old man with liver cirrhosis due to HCV genotype Ib infection. The patient's height was 168 cm, weight 70.4 kg, and body mass index 24.9.

HCV RNA was 1,200 kIU/ml. The patient had undergone IFN therapy with conventional IFN α-2b (6 MU) plus RBV (800 mg) for 24 months since 2002. He was administered IFN α-2b (6 MU) for thrombopenia, but the therapy was stopped since he showed no response to the therapy.

The patient developed hepatocellular carcinoma (HCC) and was treated by hepatic resection and had stage F3 fibrosis in September 2005. After that, IFN therapy was started with PEG-IFN α-2b (60 μg) and RBV (200 mg) in December 2005. At that time, HCV RNA was 2,400 kIU/ml. However, RBV was stopped since the patient developed itching. However, HCV RNA never reached undetectable levels. After that, HCC recurred. Therefore, the patient underwent splenectomy, and hepatectomy for HCC recurrence in August 2006. At the time, the patient showed stage F3 fibrosis. After that, IFN therapy was restarted.

Table 1. Laboratory data at the start of IFN therapy after LT

CBC	
WBC/μl	3,160
RBC/μl	4.50×10^6
Hb, g/dl	13.0
Ht, %	37.4
Plt/μl	257×10^3
Blood coagulation test	
PT, %	118
Blood chemistry	
T-bil, mg/dl	2.4
AST, IU/l	89
ALT, IU/l	45
LDH, IU/l	269
ALP, IU/l	497
γGTP, IU/l	377
TP, g/dl	6.9
Alb, g/dl	3.1
TC, mg/dl	129
TTT, U	5
ZTT, U	12
BUN, mg/dl	13
Cr, mg/dl	1.17
CRP, mg/dl	<0.2
FBS, mg/dl	267
HbA_{1c}, %	6.6
NH_3, μg/ml	47
Tumor marker	
AFP, ng/ml	27.1
HCV virus marker	
HCV RNA, kIU/ml	27,000
MELD score	6
Child-Pugh	A
aa substitution in ISDR	eight
aa70 in the core region	mutant
aa91 in the core region	wild
IL28B, genotype	GG

AFP = α-Fetoprotein; Alb = albumin; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; Cr = creatinine; CRP = C-reactive protein; FBS = fasting blood sugar level; Hb = hemoglobin; LDH = lactate dehydrogenase; Plt = platelets; PT = prothrombin time; RBC = red blood cells; T-bil = total bilirubin; TC = total cholesterol; TTT = thymol turbidity test; WBC = white blood cells; ZTT = zinc sulfate turbidity test; aa substitution in ISDR = amino acid substitutions in the IFN sensitivity-determining region.

Tumor stage was stage III [9]. Treatment with curative intent was not possible owing to the presence of multiple HCC lesions. Although the MELD score was 6 and Child-Pugh score A, his sister wished to be the donor for LT; LT was performed with informed consent in June 2007. At the time, the patient showed stage F4 fibrosis.

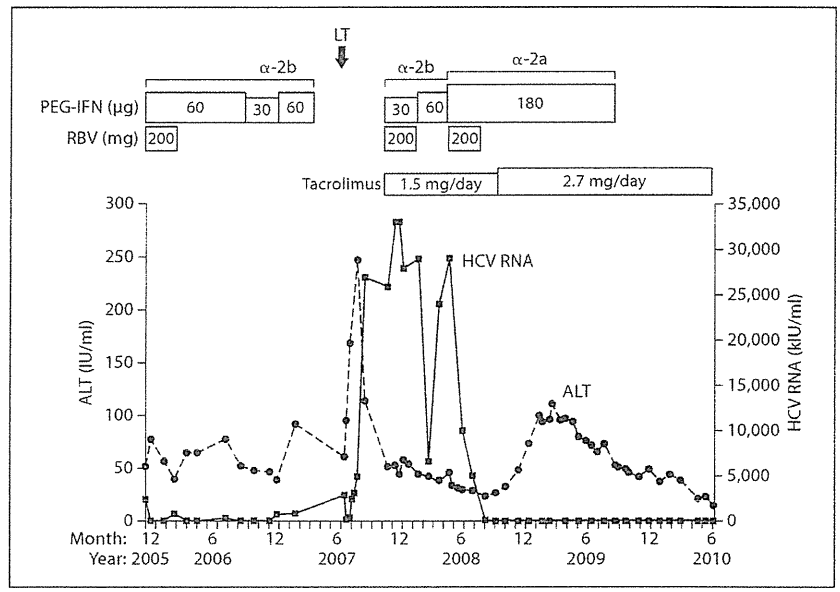

Fig. 1. Course of IFN therapy.

IFN therapy was restarted at 4 months after living donor LT in November 2007. Laboratory test data at the start of IFN therapy after LT are shown in table 1. His platelet count was 257 × 10^3/μl and ALT level 45 IU/l. Eight amino acid (aa) substitutions were detected in the IFN sensitivity-determining region (ISDR), and substitutions for mutant and wild-type were detected at aa70 and aa91, respectively, in the core region. The patient had minor genotype (GG) *IL28B* single nucleotide polymorphisms (SNPs) (rs8099917).

He was treated with PEG-IFN α-2b (60 μg) and RBV (200 mg). However, RBV administration was stopped since the patient developed itching. Although HCV RNA had decreased from 29,000 to 6,700 kIU/ml, it re-increased from 6,700 to 24,000 kIU/ml. Therefore, PEG-IFN α-2b and RBV was switched to PEG-IFN α-2a and RBV in April 2008. As a result, he was treated with PEG-IFN α-2b for 5 months.

In September 2008, 5 months after PEG-IFN α-2a and RBV, the serum HCV RNA titer became undetectable. PEG-IFN α-2a and RBV was continued until September 2009 for 12 months after the serum HCV RNA titer became undetectable, according to our protocol [7]. PEG-IFN α-2a was administered for a total of 17 months. Immunosuppressive therapy, tacrolimus 1.5 mg/day, was used at the start of IFN therapy in April 2008. Because ALT was elevated in October 2008, the dose of tacrolimus was raised up to 2.7 mg/day. As a result, ALT became normal. Finally, SVR was achieved (fig. 1).

Discussion

Recent studies have shown that various hosts and viral factors are significant predictors of the efficacy of IFN treatment. With regard to the viral factors, the number of aa substitutions in the ISDR correlated with the SVR rate in patients with HCV genotype 1b infection who underwent IFN therapy [10, 11]. Akuta et al. [12–16] reported that the substitutions at aa70 and/or aa91 in the HCV core region are independent and significant predictors of virological responses such as SVR and non-viral response to combination therapy. Our patient had 8 aa substitutions in the ISDR and substitutions for mutant and wild-type at aa70 and aa91, respectively, in the core region.

Recently, Fukuhara et al. [17] reported that mutations of the HCV core and ISDR of HCV genome were associated with the SVR rates in 50 patients. On the other hand, we reported that mutations of the HCV core and ISDR of HCV genome were not associated with the SVR rates in our previous study [7]. It was already known that IFN monotherapy for 24 weeks is enough to eradicate HCV RNA in the case of acute hepatitis C [18–20]. There was no report that HCV core mutant and substitution of aa of the ISDR region affect the SVR rate in the cases of acute hepatitis C. Since recurrence of hepatitis C in LT is thought to be another acute hepatitis C, we concluded that the mutations of the HCV core and ISDR of HCV genome do not affect the SVR rate.

Furthermore, the effect of PEG-IFN and RBV in patients with HCV genotype 1b infection is associated with several SNPs at the *IL28B* locus [21–24]. This patient had minor genotype (GG) *IL28B* SNPs (rs8099917). Recently, Fukuhara et al. [25] reported that the SVR rate

is 10–20% on minor genotype (GG) *IL28B* SNPs (rs8099917) in HCV recipients. Furthermore, Lange et al. [26] reported that the donor's *IL28B* rather than the recipient's *IL28B* affects the SVR rate. In this case the donor was his sister. Although the donor's SNP was not checked, there was a very small possibility that the sister's SNP was a major genotype (TT), since the recipient's parents had the necessary G allele in one allele of two alleles respectively.

Breakthrough viremia can be attributed to a variety of reasons. One possible cause is the development of antibodies against PEG-IFN α-2b. Vallbracht et al. [27] first reported the development of neutralizing immunoglobulin-G antibodies against natural human fibroblast IFN in a patient treated with the said IFN in 1981. Furthermore, several studies have reported neutralizing anti-IFN antibodies due to administration of IFN [28–35]. Achievement of a complete SVR in patients with HCV infection by switching the previously administered IFN with another has been reported in several studies [2, 5, 36]. Therefore, we think this patient achieved SVR by switching treatment with PEG-IFN from α-2b to α-2a. The other possible cause for the occurrence of breakthrough viremia is the generation of HCV escape mutants during IFN therapy [37]. In addition, downregulation of specific IFN cell receptors due to IFN therapy may also be a cause of breakthrough viremia [38]. Another possible cause is that the dosage of RBV was suboptimal. RBV was stopped due to itching. A further reason might be that the initial dose of PEG-IFN α-2b was insufficient. The dose of PEG-IFN α-2b was intentionally administrated at 30 μg because of the patient's general fatigue, and then the PEG-IFN dose was elevated to 60 μg. The PEG-IFN dose was going to be increased, however breakthrough viremia occurred. Therefore, we switched PEG-IFN from α-2b to α-2a and RBV.

In summary, we have reported a male patient in whom SVR was achieved by switching treatment with PEG-IFN from α-2b to α-2a and RBV for recurrence of HCV genotype 1 infection after LT. Switching an originally administered IFN with another type may be effective for the treatment of patients with HCV infection after LT.

References

1 Poynard T, Colombo M, Bruix J, Schiff E, Terg R, Flamm S, Moreno-Otero R, Carrilho F, Schmidt W, Berg T, McGarrity T, Heathcote EJ, Goncales F, Diago M, Craxi A, Silva M, Bedossa P, Mukhopadhyay P, Griffel L, Burroughs M, Brass C, Albrecht J: Peginterferon alfa-2b and ribavirin: effective in patients with hepatitis C who failed interferon alfa/ribavirin therapy. Gastroenterology 2009;136:1618–1628 e1612.

2 Leroy V, Baud M, de Traversay C, Maynard-Muet M, Lebon P, Zarski JP: Role of anti-interferon antibodies in breakthrough occurrence during alpha 2a and 2b therapy in patients with chronic hepatitis C. J Hepatol 1998;28:375–381.

3 Antonelli G, Giannelli G, Currenti M, Simeoni E, Del Vecchio S, Maggi F, Pistello M, Roffi L, Pastore G, Chemello L, Dianzani F: Antibodies to interferon (IFN) in hepatitis C patients relapsing while continuing recombinant IFN-alpha-2 therapy. Clin Exp Immunol 1996;104:384–387.

4 Awad T, Thorlund K, Hauser G, Stimac D, Mabrouk M, Gluud C: Peginterferon alpha-2a is associated with higher sustained virological response than peginterferon alfa-2b in chronic hepatitis C: systematic review of randomized trials. Hepatology 2010;51:1176–1184.

5 Herrine SK, Brown RS Jr, Bernstein DE, Ondovik MS, Lentz E, Te H: Peginterferon alpha-2a combination therapies in chronic hepatitis C patients who relapsed after or had a viral breakthrough on therapy with standard interferon alpha-2b plus ribavirin: a pilot study of efficacy and safety. Dig Dis Sci 2005;50:719–726.

6 Dinges S, Morard I, Heim M, Dufour JF, Mullhaupt B, Giostra E, Clavien PA, Mentha G, Negro F: Pegylated interferon-alpha-2a/ribavirin treatment of recurrent hepatitis C after liver transplantation. Transpl Infect Dis 2009;11:33–39.

7 Kawaoka T, Hiraga N, Takahashi S, Takaki S, Mitsui F, Tsuge M, Nagaoki Y, Kimura Y, Hashimoto Y, Katamura Y, Hiramatsu A, Waki K, Imamura M, Kawakami Y, Aikata H, Tashiro H, Ohdan H, Chayama K: Prolongation of interferon therapy for recurrent hepatitis C after living donor liver transplantation: analysis of predictive factors of sustained virological response, including amino acid sequence of the core and NS5A regions of hepatitis C virus. Scand J Gastroenterol 2010;45:1488–1496.

8 Lodato F, Berardi S, Gramenzi A, Mazzella G, Lenzi M, Morelli MC, Tame MR, Piscaglia F, Andreone P, Ballardini G, Bernardi M, Bianchi FB, Biselli M, Bolondi L, Cescon M, Colecchia A, D'Errico A, Del Gaudio M, Ercolani G, Grazi GL, Grigioni W, Lorenzini S, Pinna AD, Ravaioli M, Roda E, Sama C, Vivarelli M: Clinical trial: Peginterferon alfa-2b and ribavirin for the treatment of genotype-1 hepatitis C recurrence after liver transplantation. Aliment Pharmacol Ther 2008;28:450–457.

9 Liver Cancer Study Group of Japan: The general rules for the clinical and pathological study of primary liver cancer. Jpn J Surg 1989;19:98–129.

10 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C: Mutations in the nonstructural protein 5a gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996;334:77–81.

11 Chayama K, Tsubota A, Kobayashi M, Okamoto K, Hashimoto M, Miyano Y, Koike H, Koida I, Arase Y, Saitoh S, Suzuki Y, Murashima N, Ikeda K, Kumada H: Pretreatment virus load and multiple amino acid substitutions in the interferon sensitivity-determining region predict the outcome of interferon treatment in patients with chronic genotype 1b hepatitis C virus infection. Hepatology 1997;25:745–749.

12 Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Arase Y, Ikeda K, Kumada H: Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 2005;48:372–380.

13 Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Arase Y, Ikeda K, Kumada H: Predictive factors of virological non-response to interferon-ribavirin combination therapy for patients infected with hepatitis C virus of genotype 1b and high viral load. J Med Virol 2006;78:83–90.
14 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Arase Y, Ikeda K, Kumada H: Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. J Med Virol 2007;79:1686–1695.
15 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Arase Y, Ikeda K, Kumada H: Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 2007;46:403–410.
16 Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H: A matched case-controlled study of 48 and 72 weeks of peginterferon plus ribavirin combination therapy in patients infected with HCV genotype 1b in Japan: amino acid substitutions in HCV core region as predictor of sustained virological response. J Med Virol 2009;81:452–458.
17 Fukuhara T, Taketomi A, Okano S, Ikegami T, Soejima Y, Shirabe K, Maehara Y: Mutations in hepatitis C virus genotype 1b and the sensitivity of interferon-ribavirin therapy after liver transplantation. J Hepatol 2010;52:672–680.
18 Ogata K, Ide T, Kumashiro R, Kumada H, Yotsuyanagi H, Okita K, Akahane Y, Kaneko S, Tsubouchi H, Tanaka E, Moriwaki H, Nishiguchi S, Kakumu S, Mizokami M, Iino S, Sata M: Timing of interferon therapy and sources of infection in patients with acute hepatitis C. Hepatol Res 2006;34:35–40.
19 Kamal SM, Fouly AE, Kamel RR, Hockenjos B, Al Tawil A, Khalifa KE, He Q, Koziel MJ, El Naggar KM, Rasenack J, Afdhal NH: Peginterferon alfa-2b therapy in acute hepatitis C: impact of onset of therapy on sustained virologic response. Gastroenterology 2006;130:632–638.
20 Delwaide J, Bourgeois N, Gerard C, De Maeght S, Mokaddem F, Wain E, Bastens B, Fevery J, Gehenot M, Le Moine O, Martinet JP, Robaeys G, Servais B, Van Gossum M, Van Vlierberghe H: Treatment of acute hepatitis C with interferon alpha-2b: early initiation of treatment is the most effective predictive factor of sustained viral response. Aliment Pharmacol Ther 2004;20:15–22.
21 Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB: Genetic variation in *IL28B* predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.
22 Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Muller T, Bahlo M, Stewart GJ, Booth DR, George J: *IL28B* is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009;41:1100–1104.
23 Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M: Genetic variation in *IL28B* and spontaneous clearance of hepatitis C virus. Nature 2009;461:798–801.
24 Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M: Genome-wide association of *IL28B* with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105–1109.
25 Fukuhara T, Taketomi A, Motomura T, Okano S, Ninomiya A, Abe T, Uchiyama H, Soejima Y, Shirabe K, Matsuura Y, Maehara Y: Variants in *IL28B* in liver recipients and donors correlate with response to peginterferon and ribavirin therapy for recurrent hepatitis C. Gastroenterology 2010;139:1577–1985, 1585.e1-3.
26 Lange CM, Moradpour D, Doehring A, Lehr HA, Mullhaupt B, Bibert S, Bochud PY, Antonino AT, Pascual M, Farnik H, Shi Y, Bechstein WO, Moench C, Hansmann ML, Sarrazin C, Lotsch J, Zeuzem S, Hofmann WP: Impact of donor and recipient *IL28B* rs12979860 genotypes on hepatitis C virus liver graft reinfection. J Hepatol 2010 (E-pub ahead of print).
27 Vallbracht A, Treuner J, Flehmig B, Joester KE, Niethammer D: Interferon-neutralizing antibodies in a patient treated with human fibroblast interferon. Nature 1981;289:496–497.
28 Porres JC, Carreno V, Ruiz M, Marron JA, Bartolome J: Interferon antibodies in patients with chronic HBV infection treated with recombinant interferon. J Hepatol 1989;8:351–357.
29 Jones GJ, Itri LM: Safety and tolerance of recombinant interferon alfa-2a (Roferon-a) in cancer patients. Cancer 1986;57:1709–1715.
30 Antonelli G, Currenti M, Turriziani O, Dianzani F: Neutralizing antibodies to interferon-alpha: relative frequency in patients treated with different interferon preparations. J Infect Dis 1991;163:882–885.
31 Arai K, Shindo M, Okuno T: Anti-interferon antibody in chronic hepatitis C (in Japanese). Nippon Rinsho 1994;52:1929–1934.
32 Bonetti P, Diodati G, Drago C, Casarin C, Scaccabarozzi S, Realdi G, Ruol A, Alberti A: Interferon antibodies in patients with chronic hepatitic C virus infection treated with recombinant interferon alpha-2 alpha. J Hepatol 1994;20:416–420.
33 Milella M, Antonelli G, Santantonio T, Currenti M, Monno L, Mariano N, Angarano G, Dianzani F, Pastore G: Neutralizing antibodies to recombinant alpha-interferon and response to therapy in chronic hepatitis C virus infection. Liver 1993;13:146–150.
34 Hou C, Chuang WL, Yu ML, Dai CY, Chen SC, Lin ZY, Hsieh MY, Wang LY, Tsai JF, Chang WY: Incidence and associated factors of neutralizing anti-interferon antibodies among chronic hepatitis C patients treated with interferon in Taiwan. Scand J Gastroenterol 2000;35:1288–1293.
35 Boo I, Fischer AE, Johnson D, Chin R, Giourouki M, Bharadwaj M, Bowden S, Torresi J, Drummer H: Neutralizing antibodies in patients with chronic hepatitis C infection treated with (PEG)-interferon/ribavirin. J Clin Virol 2007;39:288–294.
36 Roffi L, Mels GC, Antonelli G, Bellati G, Panizzuti F, Piperno A, Pozzi M, Ravizza D, Angeli G, Dianzani F, et al: Breakthrough during recombinant interferon alfa therapy in patients with chronic hepatitis C virus infection: prevalence, etiology, and management. Hepatology 1995;21:645–649.
37 Okada S, Akahane Y, Suzuki H, Okamoto H, Mishiro S: The degree of variability in the amino terminal region of the E2/NS1 protein of hepatitis C virus correlates with responsiveness to interferon therapy in viremic patients. Hepatology 1992;16:619–624.
38 Nakajima S, Kuroki T, Shintani M, Kurai O, Takeda T, Nishiguchi S, Shiomi S, Seki S, Kobayashi K: Changes in interferon receptors on peripheral blood mononuclear cells from patients with chronic hepatitis B being treated with interferon. Hepatology 1990;12:1261–1265.

ELSEVIER

Optimization of Immunosuppressive Therapy Based on a Multiparametric Mixed Lymphocyte Reaction Assay Reduces Infectious Complications and Mortality in Living Donor Liver Transplant Recipients

Y. Tanaka, H. Tashiro, T. Onoe, K. Ide, K. Ishiyama, and H. Ohdan

ABSTRACT

Aim. We investigated the clinical relevance of immune monitoring by a multiparametric mixed lymphocyte reaction (MLR) assay, wherein the number and phenotype of alloreactive precursors can be quantified by combining the results of carboxyfluorescein diacetate succinimidyl ester labeling and flow cytometry analysis.

Methods. In 51 adult patients undergoing living donor liver transplantation (OLT), immunosuppressive drugs were dosed on the basis of immune monitoring by the MLR assay (optimized protocol: group O). In 64 other patients, the agents were prescribed according to empirical regimens (empirical protocol: group E). In group O, MLR assays were performed at 2- to 4-week intervals until 3 months after OLT and thereafter at 3- to 6-month intervals. Therapeutic adjustments for immunosuppressants were determined by tapering the doses in cases of anti-donor hyporesponsiveness for both $CD4^+$ and $CD8^+$ T-cell subsets.

Results. The 1-year patient and graft survivals in groups O versus E were 90.2% versus 76.6%, respectively. The incidence of acute rejection episodes (ARE) among group O (13.7%) were lower than in cohort E (28.1%). None of the patients in group O while four patients (3%) in group E already have shown chronic rejection to date. The incidences of bacteremia and fungal infections in group O (9.8% and 7.5%, respectively) were lower than in cohort E (18.8% and 12.6%, respectively).

Conclusion. A multiparametric MLR assay may facilitate the development of adequate immunosuppressive regimens.

PATIENTS UNDERGOING LIVER TRANSPLANTATION (OLT) receive immunosuppressants according to empirical protocols, which seek to take into account the risks of under- versus oversuppression, rejection, infection, and adverse drug reactions. However, an individually optimized immunosuppressive protocol developed on the basis of immune monitoring would be useful to avoid these undesirable effects. The aim of this study was to investigate the clinical relevance of immune monitoring by a multiparametric mixed lymphocyte reaction (MLR) assay, wherein the number and phenotype of alloreactive precursors is quantified by combining the results of carboxyfluorescein diacetate succinimidyl ester (CFSE) labeling with flow cytometry (FCM) analysis.

PATIENTS AND METHODS

Patient Population and Immunosuppressive Protocol

We enrolled 115 patients who underwent adult-to-adult living donor OLT (LDLT). The basic immunosuppressive regimen consisted of tacrolimus or cyclosporine with methylprednisolone in gradually tapered doses. Among 64/115 patients, the immunosuppressants were dosed according to empirical regimens (empirical protocol: group E). In 51 other patients, immunosuppressive drugs were prescribed on the basis of immune monitoring by the MLR assay (optimized protocol: group O). In this group, MLR assays were performed at 2- to 4-week intervals until 3 months after LDLT, and thereafter at intervals of 3 to 6 months.

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MLR Assay

Peripheral blood mononuclear cells (PBMCs) prepared from recipients (autologous controls), donors or healthy volunteers (third-party control) serving as stimulator cells were irradiated with 30 Gy. Recipient responder cells were labeled with CFSE. Both the stimulator and responder cells were cocultured at 37°C in the dark for 5 days, as described previously.[1] After MLR culture, harvested nonadherent cells were stained with either phycoerythrin-conjugated CD4 or CD8 monoclonal antibodies (mAbs; BD Pharmingen, San Diego, Calif, USA) together with allophycocyanin-conjugated CD25 mAb (BD Pharmingen). The four-color FCM was performed on a FACSCalibur dual-laser cytometer (Becton Dickinson, Mountain View, Calif, USA). Dead cells were excluded from the analysis by light scatter and/or propidium iodide fluorescence.

Quantifying Proliferation of $CD4^+$ and $CD8^+$ T Cells

Precursor frequency (PF), proliferation index (PI), and stimulation index (SI) were quantitatively estimated using the previously described method.[1]

In brief, divisions of reactive T cells, which were identified by their CFSE intensities, labeled from 0 to n were the dividing time. A single cell dividing n times generates 2^n daughter cells. Using this mathematical relationship, the number of division precursors was extrapolated from the number of daughter cells of each division and from proliferation events and PF in $CD4^+$ and $CD8^+$ T-cell subsets. Using these values, proliferation events and PIs were calculated. The SI was calculated by dividing the PIs of allogeneic combinations by those of autologous controls.

Adjustments for Immunosuppressants

On the basis of the proliferation analysis of $CD4^+$ and $CD8^+$ T-cell subsets in response to anti-donor versus anti-third party stimuli in MLR, we categorized the immune status as hypo-, normo-, or hyperresponsive (Fig 1). Therapeutic adjustments for immunosuppressants were determined by tapering dosages in cases exhibiting anti-donor hyporesponsiveness in both T-cell subsets or by increasing them for anti-donor hyperresponsiveness.

Statistical Analysis

For continuous variables, parametric analyses were performed using Student t test, and the Mann-Whitney U test for nonparametric analyses. Categorical variables and postoperative courses were compared using χ^2 tests with Yates correction. For factors determined to be significant for survival rates, as well as incidences of infection and ARE upon univariate analysis, we performed multivariate analyses using logistic regression. A difference was considered significant if the

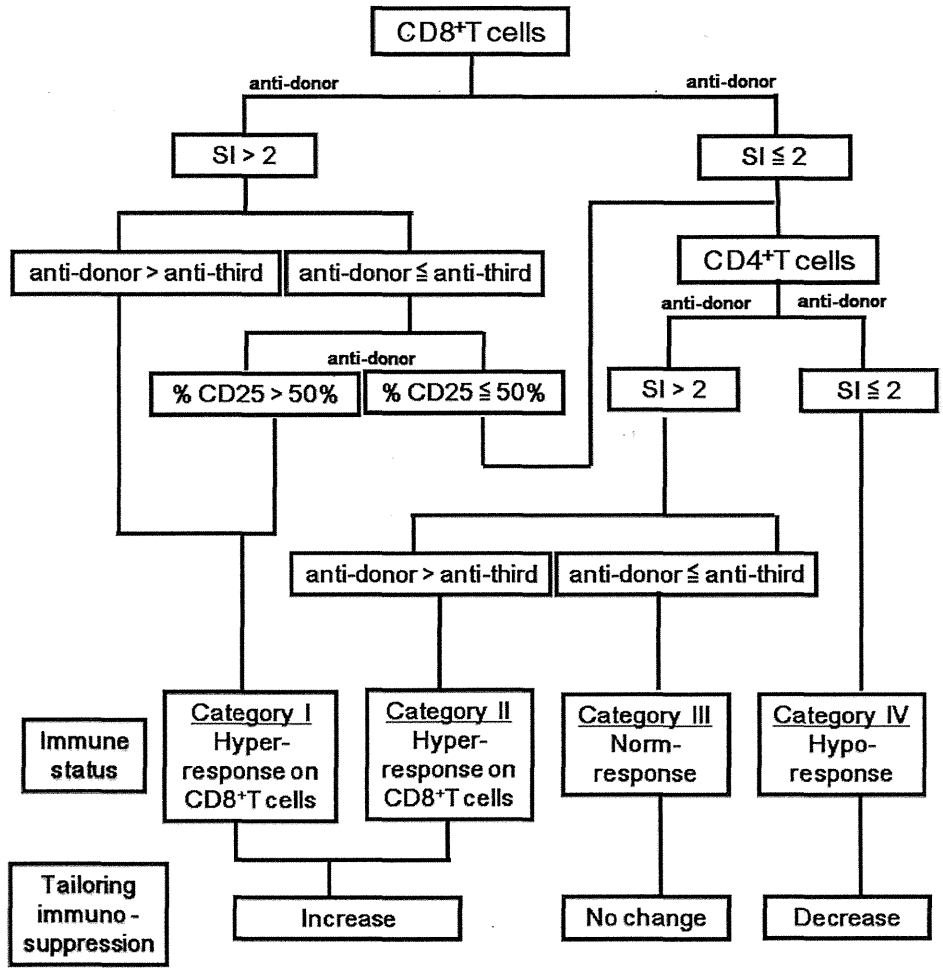

Fig 1. Algorithm to determine anti-donor alloreactivity in liver transplant patients. The immune status of liver transplantation patients was classified into four categories. By analyzing the proliferation and CD25 expression for the $CD4^+$ and $CD8^+$ T-cell subsets in response to anti-donor and anti-third party stimuli, the immune status was categorized as hypo-, normo-, or hyperresponsive. In patients with hyperresponsive immune status on either $CD4^+$ or $CD8^+$ T cells, immunosuppressants were increased. In patients with normoresponsive immune status, immunosuppressant tapering was abandoned. Only in patients with hyporesponsive immune status, immunosuppressant therapy was tapered off. SI, stimulation index.

Table 1. Patient Profile

	Emprical Group (group E)	Optimized Group (group O)	*P* Value
Case number	64	51	
Age	51.2 ± 10.7	54.1 ± 9.4	.12
Sex (M vs F)	35 vs 29	21 vs 30	.23
MELD score	19.2 ± 9.1	15.4 ± 6.4	.002
Donor aging	35.0 ± 12.4	37.1 ± 12.5	.36
Relationship of donor and recipient (blood relationship vs non-blood relationship)	11 vs 53	7 vs 54	.61
Child classification (A vs B vs C)	8 vs 21 vs 35	11 vs 17 vs 23	.38
GRWR	0.99 ± 0.25	0.97 ± 0.27	.72
Operation time (min)	709.7 ± 113.2	750.2 ± 139.8	.09
Blood loss during surgery (mL)	4501.5 ± 3596.0	4416.3 ± 2703.6	.89
Original disease (acute liver failue vs chronic liver failue)	7 vs 57	1 vs 50	.06

MELD, Model for End-stage Liver Disease; GRWR, graft-recipient body weight ratio.

P value was less than .05. Statistical analyses were performed using the SPSS statistical software version 16 (Chicago, Ill, USA).

RESULTS

The patient profiles are shown in Table 1. The Model for End-stage Liver Disease score in group E was higher than that in group O, probably reflecting the greater proportion of patients with acute liver failure as an original disease in group E compared with group O. There were no differences in other background factors between the groups. Figure 2 shows the change in immune status determined by the MLR assay during the observation period. The proportion of patients showing anti-donor hyporesponsiveness gradually increased over time. At 3 to 6 months after transplantation, immunosuppressants were reduced in approximately 60% of group O patients. We also investigated the incidence of infectious disease within 1 year after OLT. In group O, the incidences of sepsis and fungal, bacterial, or cytomegalovirus infection were lower than those in group E, although the difference did not reach significance ($0.05 < P < 0.1$, Fisher exact test; Fig 3A). The incidence of ARE in the O cohort was also lower than that in group E (Fig 3B). Furthermore, none of the patients in group O showed chronic rejection versus 3% in group E (Fig 3B). The 6- and 12-month patient survival rates in group O were a bit higher than those in group E, although the difference did not reach significance (Fig 3C). Univariate analyses of differences in the incidences of ARE and infection, as well as 6- and 12-month survival rates between the groups are shown in Table 2. Multivariate analyses to determine factors associated with these metrics showed only donor age to be significantly associated with 1-year survival rates (Table 3).

An additional benefit of optimizing immunosuppression under the regimen of immune monitoring would be a

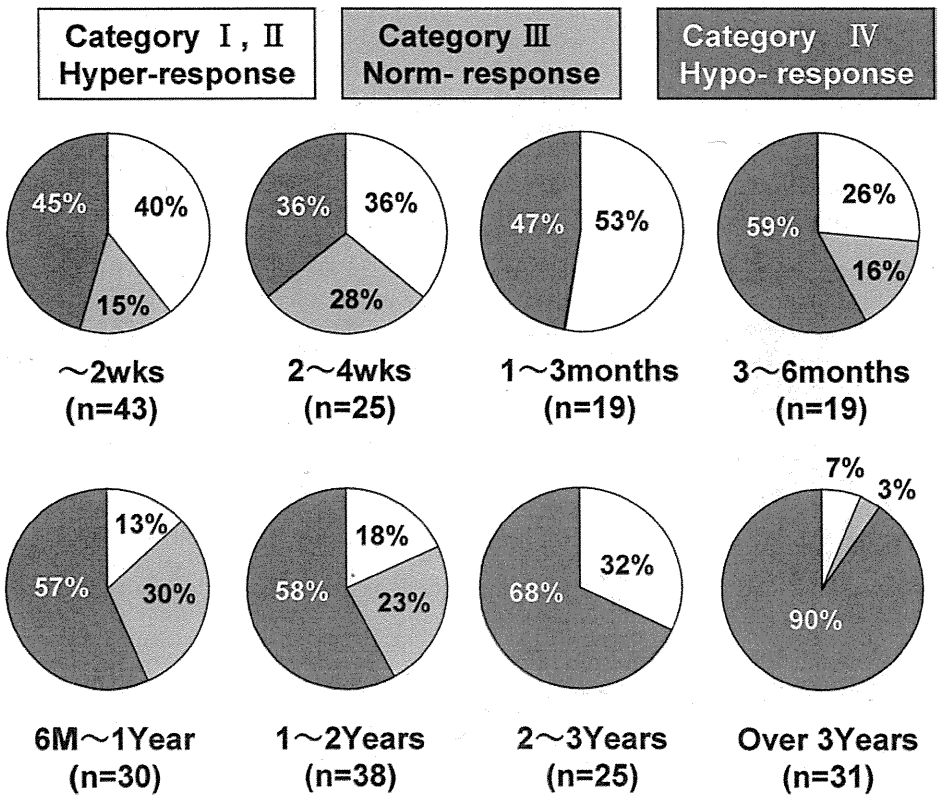

Fig 2. Change in immune status after liver transplantation. The immune status of liver transplantation patients was classified into four categories. By analyzing the proliferation and CD25 expression for the $CD4^+$ and $CD8^-$ T-cell subsets in response to anti-donor and anti-third-party stimuli, the immune status was categorizedas hypo-, normo-, or hyperresponsive. The proportions of patients categorized into each immune status at various times after liver transplantation are shown.

successful response to vaccination to prevent viral infections. For instance, hepatitis B virus (HBV) vaccination can prevent reinfection with HBV. However, the immunosuppressive environment is believed to result in a poor response to vaccination.[2] We observed that the overall response rate to HBV vaccination among group O was higher than cohort E, suggesting that minimized immunosuppression may enable posttransplant HBV vaccination, a promising prophylactic strategy (Fig 4).

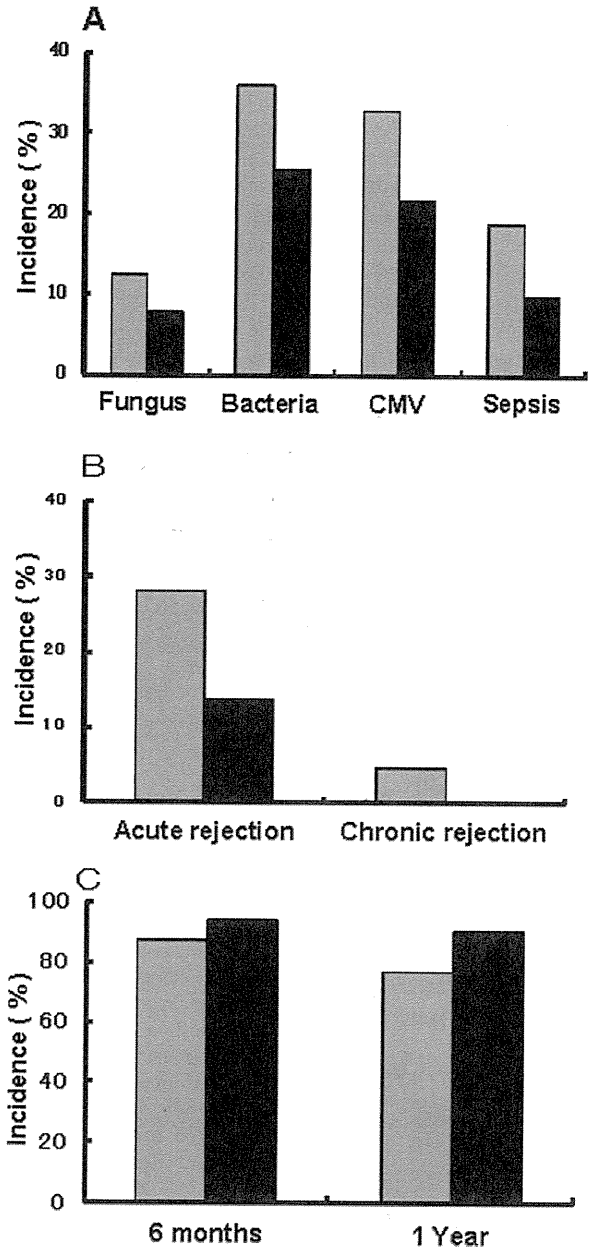

Fig 3. Incidences of infectious disease, acute rejection, and chronic rejection within 1 year after liver transplantation. The incidences of infectious disease **(A)**, acute rejection, and chronic rejection **(B)** within 1 y after liver transplantation were investigated. In addition, the 6-month and 1-year patient survival rates in each group are shown **(C)**. CMV, cytomegalovirus.

Table 2. Results of Univariate Analyses to Determine the Difference in the Incidence of Rejection and Infection and 6-mo and 1-y Survival Between Empirical Group and Optimized Group

	P
Incidence of acute rejection	.055
Incidence of infection	.776
6-mo survival	.231
1-y survival	.107

DISCUSSION

Anti-donor alloreactivity, defined as the number and phenotype of alloreactive precursors in the recipient, can be used to monitor graft rejection, to guide treatment to reduce ARE, or to withdraw immunosuppression. MLR using PBMCs is widely used in both experimental and clinical transplantation to evaluate T-cell responses to allogeneic stimulation. However, conventional assays of MLR using tritiated thymidine incorporation show little predictive value because of their low level of reproducibility.[3] This limitation might be caused—at least in part—by the presence of nonviable cells (which might include unexpectedly surviving stimulator cells) that retain the ability to incorporate tritiated thymidine. With a CFSE-based method, the proliferation of viable $CD4^+$ and $CD8^+$ responder T cells in response to allostimulation is quantified separately using a multiparameter FCM. A lack of proliferation by both $CD4^+$ and $CD8^+$ T cells in anti-donor MLR may reflect effective suppression of the anti-donor responses. When remarkable proliferation was observed among $CD4^+$ but not in $CD8^+$ T cells, we did not observe cytotoxic activity against donor cells in subsequent cell-mediated lympholysis assays in our previous studies.[4] In contrast, remarkable proliferation of $CD8^+$ T cells reflect strong anti-donor responses. We further examined CD25 expression on the proliferating $CD8^+$ T cells by multicolor FCM. In our previous studies, a remarkable elevation of CD25 expression on proliferating $CD8^+$ T cells was observed to reflect their cytotoxic activity toward donor cells.[4] By using such a multiparametric MLR assay, we demonstrated that a careful evaluation of recipient immune status facilitated the development of adequate immunosuppressive regimens. Optimization of immunosuppression in this manner seems to be a promising strategy to reduce infectious complications and mortality among patients undergoing LDLT.

Table 3. Multivariate Analysis of 1-y Survival

Variable	Hazard Ratio	95% Confidence Interval	*P* Value
Donor age	0.959	0.923–0.997	.033

REFERENCES

1. Tanaka Y, Ohdan H, Onoe T, et al: Multiparameter flow cytometric approach for simultaneous evaluation of proliferation and cytokine-secreting activity in T cells responding to allostimulation. Immunol Invest 33:309, 2004

2. Tahara H, Tanaka Y, Ishiyama K, et al: Successful hepatitis B vaccination in liver transplant recipients with donor-specific hyporesponsiveness. Transpl Int 22:805, 2009

3. Segall M, Noreen H, Edwins L, et al: Lack of correlation of MLC reactivity with acute graft-versus-host disease and mortality in unrelated donor bone marrow transplantation. Hum Immunol 49:49, 1996

4. Tanaka Y, Ohdan H, Onoe T, et al: Low incidence of acute rejection after living-donor liver transplantation: immunologic analyses by mixed lymphocyte reaction using a carboxyfluorescein diacetate succinimidyl ester labeling technique. Transplantation 79:1262, 2005

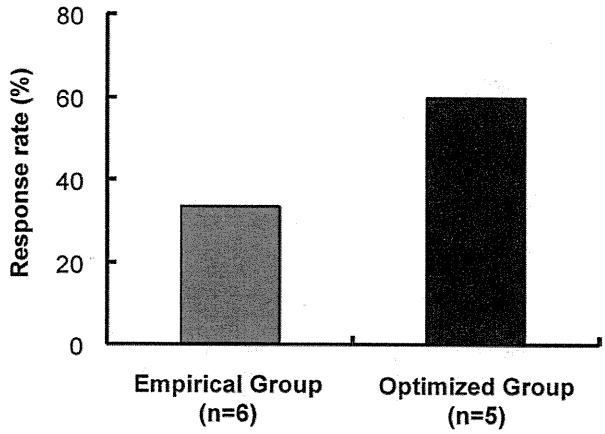

Fig 4. Overall response rate to hepatitis B virus (HBV) vaccination within 1 year after commencing HBV vaccination. All participants received a yeast-derived recombinant adsorbed HBV vaccine (Bimmugen) subcutaneously every 4 weeks at a dose of 10 to 20 μg (0.5–1.0 mL) in combination with HBIg and lamivudine/adefovir. HBIg immunoprophylaxis was continued during primary immunization (dose, 1000–2000 IU every 4 weeks). The response to vaccination was defined as (1) a confirmed increase in the anti-hepatitis B surface (HBs) antigen HBs titer to >100 IU/L that could not be explained by HBIg administration and (2) sustained anti-HBs titer to >100 IU/L after discontinuation of combined administration of the vaccine and HBIg. If the anti-HBs titer exceeded the responsive increasing level, HBIg substitution and vaccine administration were discontinued. Lamivudine/adefovir prophylaxis was additionally discontinued, if the anti-HBs titer was maintained effectively without HBIg administration. The vaccine was continuously and indefinitely administered till acquired immunity was elicited.

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ORIGINAL ARTICLE – HEPATOBILIARY TUMORS

Impact of Pegylated Interferon Therapy on Outcomes of Patients with Hepatitis C Virus-Related Hepatocellular Carcinoma After Curative Hepatic Resection

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ABSTRACT

Background. Several published reports investigating the effects of interferon (IFN) therapy on survival and tumor recurrence after curative resection of hepatocellular carcinoma (HCC) have been inconclusive. The aim of this study is to investigate the efficacy of pegylated-IFN (peg-IFN) therapy after curative hepatic resection for HCC in patients infected with hepatitis C virus (HCV).

Methods. Data from 175 patients who underwent curative hepatic resection for HCC associated with HCV were retrospectively collected and analyzed; 75 patients received peg-IFN therapy after surgery, whereas 100 patients did not receive IFN therapy. To overcome biases resulting from the different distribution of covariates in the two groups, a one-to-one match was created using propensity score analysis. After matching, patient outcomes were analyzed.

Results. After one-to-one matching, patients ($n = 38$) who received peg-IFN therapy after surgery and patients ($n = 38$) who did not receive IFN therapy had the same preoperative and operative characteristics. The 3- and 5-year overall survival rates of patients who received peg-IFN therapy after hepatic resection were significantly higher than those of patients who did not receive IFN therapy ($P = 0.00135$). The 3- and 5-year overall survival rates were 100 and 91.7% and 76.6 and 50.6% in the peg-IFN group and non-IFN group, respectively. There was no significant difference in disease-free survival between the two matched groups ($P = 0.886$).

Conclusion. Peg-IFN therapy may be effective as an adjuvant chemopreventive agent after hepatic resection in patients with HCV-related HCC.

Hepatic resection is a well-accepted therapy for hepatocellular carcinoma (HCC), but many patients show cancer recurrence and the cumulative 5-year HCC recurrence rate exceeds 70%.[1–3] This high incidence of tumor recurrence after hepatic resection remains a major drawback. Some benefits of interferon (IFN) therapy on tumor recurrence and survival have been reported.[4–10] IFN suppresses replication of hepatitis C virus (HCV) and exerts a tumoricidal effect on a number of tumors, including HCC.[10,11] However, several randomized controlled trials (RCTs) have revealed inconclusive results regarding the effects of IFN on survival and tumor recurrence after curative resection or ablation of HCC, either because the effects were not statistically significant or because they were considered only with respect to defined subpopulations.[12–15]

Recently, combination therapy consisting of pegylated interferon (peg-IFN) plus ribavirin (RBV) has been developed, and the effect of this combination has been reported to be higher than that of conventional IFN therapy.[16,17] Peg-IFN has an extended serum half-life that provides viral suppression for 7 days, thus allowing weekly administration and enhanced clinical efficacy.[17] Most Japanese patients infected with HCV are infected with HCV genotype Ib and have high viral load. Moreover, treatment with conventional IFN is complicated by a low sustained viral response (SVR) rate of 20–30%.[18–20]

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However, peg-IFN plus RBV combination therapy has good tolerability in Japanese patients with HCV and resulted in an SVR rate of approximately 40–50%.[21–23] The impact of adjuvant immunotherapy with IFN after curative resection of HCC is debatable, and few studies have investigated the effects of peg-IFN plus RBV combination therapy on survival and recurrence after curative resection of HCC.

In the present study, we aim to investigate the impact of peg-IFN plus RBV combination therapy on survival and HCC recurrence after curative resection in patients infected with HCV.

PATIENTS AND METHODS

Patients and HCV Diagnosis

From June 2003 to June 2009, 370 HCC patients underwent hepatectomy as initial treatment at the Department of Gastroenterological Surgery, Hiroshima University Hospital, Japan. Of the 370 patients, 175 patients who were HCV RNA-positive/hepatitis B surface antigen-negative underwent curative hepatectomy. Of the 175 patients, 75 patients received IFN therapy after hepatectomy, and 100 patients did not receive any IFN therapy. Of the 75 patients who received IFN, 20 patients who received IFNs such as IFN-α or IFN-β were excluded. Of the 55 patients who received peg-IFN therapy, 43 patients who started peg-IFN within 9 months after curative resection were enrolled in this analysis. Twenty-four patients who had early recurrence of HCC within 9 months after surgery were excluded from the 100 patients who did not receive any IFN therapy, because these patients could lose the opportunity to receive IFN therapy for HCC recurrence if these patients were assigned to the peg-IFN therapy. Consequently, 119 patients were eventually enrolled in this study. Of these 119 patients, 43 received peg-IFN therapy within 9 months after hepatectomy, and 76 did not receive any IFN therapy.

Curative hepatectomy was defined as removal of all recognizable tumors. HCV RNA levels were measured by quantitative reverse-transcription polymerase chain reaction (RT-PCR; Amplicor, Roche Diagnostic Systems, CA, USA). HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of the NS5 region. HCV negativity was evaluated by quantitative RT-PCR. The lower limit of the assay was 5 kIU/ml (equivalent to 5,000 copies/ml) in the quantitative method and 50 IU/ml (equivalent to 50 copies/ml) in the qualitative method. SVR was defined as undetectable HCV RNA at 24 weeks after completion of IFN therapy. The study was approved by the concerned institutional review boards. Written informed consent was obtained from all patients.

Preoperative Diagnosis and Evaluation of HCC

Hepatocellular carcinoma was diagnosed on the basis of routine imaging modalities such as Doppler ultrasonography (US), computed tomography (CT) during hepatic angiography (CTHA) and CT during arterial portography (CTAP), and magnetic resonance imaging. Tumor stage, liver damage classification, and surgical procedures were defined according to the General Rules for Clinical and Pathologic Study of Primary Liver Cancer, fifth edition, by the Liver Cancer Study Group of Japan.[24]

Hepatectomy

The surgical procedure was determined according to tumor extent and hepatic reserve function. Liver function was assessed by liver damage classification, Child–Pugh classification, and indocyanine green retention rate at 15 min (ICGR 15).[25,26] If permitted by liver function, anatomic resection was performed.[27,28] In patients with insufficient hepatic reserve, limited resection was performed. We divided the liver parenchyma by using an ultrasonic dissector.[29] Postoperative complications were graded according to the method described by Clavien et al.[30]

Follow-Up

Follow-up evaluation after the surgery consisted of monthly blood chemistry tests and measurements of levels of tumor markers, including alpha-fetoprotein and des-gamma-carboxy prothrombin. Patients were examined by US every 3 months and by CT every 6 months. When recurrence was indicated by any of these examinations, patients were examined by CTAP and CTHA.

Patient Selection for IFN Therapy

Patients with HCV genotype 1b in the IFN group received peg-IFNα-2b (Pegintron; Schering-Plough, NJ, USA) at weekly dosage of 1.5 μg/kg subcutaneously for 48 weeks. Daily RBV (Rebetrol, Schering-Plough) was administered orally for 48 weeks, and the dosage was adjusted according to weight (600 mg for patients weighing $\leq$60 kg, 800 mg for those weighing 60–80 kg). Patients with HCV genotype 2 received IFN monotherapy for 24 weeks. Blood samples were obtained every 4 weeks and analyzed for HCV RNA levels. All patients were informed about IFN therapy after hepatectomy, and only consenting patients received IFN therapy. The eligibility criteria for IFN therapy were as follows: (1) detectable serum HCV RNA level, (2) Eastern Cooperative Oncology

Group (ECOG) performance score of 0 or 1, (3) platelet count $\geq$70,000/μl, (4) patients with no uncompensated cirrhosis (Child class C), and (5) hemoglobin concentration $\geq$10 g/dl. Peg-IFN therapy was commenced within 24 weeks of surgery or after the eligibility criteria were fulfilled.

Safety Assessments and Dose Modification of Peg-IFN Therapy

Adverse events were graded as mild, moderate, severe, or potentially life-threatening according to a modified World Health Organization grading system. The dose of peg-IFN was decreased by 50% and that of RBV was lowered to half in case of severe adverse events or when laboratory results revealed any of the following: hemoglobin concentration <10 g/dl in patients with no cardiac disease, decrease in hemoglobin concentration >2 g/dl in patients with cardiac disease, white blood cell count <3,000/mm^3, or platelet count <50,000/mm^3. Full dosage could be resumed on resolution of the adverse events. Treatment was permanently discontinued in case of life-threatening events or when laboratory results revealed hemoglobin concentration <7.5 g/dl after 4 weeks of dose reduction, white blood cell count <1,500/mm^3, or platelet count <30,000 mm^3.

Treatment for Recurrence

Patients with intrahepatic HCC recurrence were managed with ablative therapies such as radiofrequency ablation (RFA), percutaneous ethanol injection therapy, transarterial chemoembolization, or surgery including living-donor liver transplantation according to the tumor characteristics (number, size, and location of the tumors) and liver function.

Statistical Analyses

Categorical variables were compared using the chi-square test, and continuous variables were compared using the Mann–Whitney *U*-test. Overall survival and disease-free survival analyses were performed using Kaplan–Meier methods; comparisons between different groups were performed using the log-rank test. *P* value of less than 0.05 was considered significant. Calculations were performed using SPSS software (version 16; SPSS Inc., IL, USA).

Propensity analysis was performed using logistic regression to create a propensity score for the IFN and non-IFN therapy groups.[31,32] Variables entered in the propensity model were age, sex, HCV genotype, liver function test, tumor factors, and operative factors. The model was then used to provide a one-to-one match between the two groups by using the nearest-neighbor matching method.[33,34] Survival and disease-free survival analyses were performed in each matched subgroup to assess the impact of peg-IFN therapy on mortality after adjusting for the confounding factors.

RESULTS

Characteristics and Postoperative Course of the Entire Population

Differences in the characteristics of patients who received peg-IFN therapy after hepatic resection and those who did not receive IFN therapy after hepatic resection are presented in Table 1. Patients who received peg-IFN therapy were younger (65 vs. 71 years; $P = 0.0003$). Regarding tumor characteristics, there was no significant difference between the two groups. Operation times tended to be longer in patients who received peg-IFN therapy than in those who did not receive IFN therapy (260 vs. 242 min; $P = 0.05$). There were no hospital-related deaths in this study. Postoperative complications did not differ between the two groups. In the entire population, the 3- and 5-year overall survival rates of patients who received peg-IFN therapy after hepatic resection were significantly higher than those of patients who did not receive IFN therapy ($P = 0.0024$) (Fig. 1a). However, there was no significant difference in disease-free survival between the two groups ($P = 0.795$) (Fig. 1b).

Results After Propensity Score Matching

Characteristics of the patients after propensity score analysis are presented in Table 1. Thirty-eight of the 43 patients who received peg-IFN therapy after hepatic resection and an equal number of the 76 patients who did not receive IFN therapy were matched after covariate adjustment. The study group of 76 patients was well matched; in particular, all covariates that significantly affected recurrence and postoperative liver failure in the entire study group were equally distributed between the two matched groups. Matched patients who received peg-IFN therapy after hepatic resection had similar total bilirubin and serum albumin levels and similar platelet counts to matched patients who did not receive IFN therapy. Similarly, the tumor characteristics, the surgical procedure, operation times, and blood loss during the operation in matched patients who received peg-IFN therapy were almost similar to those in patients who did not receive IFN therapy. There were no hospital-related deaths in the matched groups. Postoperative complications also did not differ between the two groups. The median follow-up period for patients who received peg-IFN and those who

TABLE 1 Baseline characteristics and operative data on patients who underwent hepatectomy: data are reported for whole study and for the matched study population after propensity score analysis

	Overall series		*P* value	Propensity-matched series		*P* value
	IFN (+) $n = 43$	IFN (−) $n = 76$		Peg-IFN (+) $n = 38$	IFN (−) $n = 38$	
Age (years)	65 (53–78)	71 (48–83)	0.0003	65.5 (53–75)	69 (51–80)	0.2
Sex (male/female)	27/16	47/29	0.918	23/15	25/13	0.634
Preoperative IFN	24 (55.8%)	29 (38.1%)	0.06	20 (52.6%)	14 (36.8%)	0.16
HCV genotype			0.876			0.6
1b	34	61		29	27	
2b	9	15		9	11	
Diabetes mellitus	11 (25.6%)	22 (28.9%)	0.856	11 (28.9%)	13 (34.2%)	0.621
ECOG PS			0.831			0.644
0	39	68		36	35	
1	4	8		2	3	
Platelet (104/mm^3)	10.3 (3.3–26.6)	10.3 (3.8–40.3)	0.381	9.75 (3.3–21.5)	11.2 (3.8–40.3)	0.454
T-Bil (mg/dl)	0.7 (0.3–1.4)	0.8 (0.3–1.7)	0.292	0.7 (0.4–1.4)	0.7 (0.3–1.7)	0.798
AST (IU/l)	42 (18–121)	48 (16–150)	0.152	43.5 (18–127)	41.5 (6–150)	0.567
ALT (IU/l)	38 (13–127)	41.5 (10–196)	0.987	40.5 (11–127)	37.5 (10–196)	0.226
Albumin (g/dl)	3.8 (2.8–5.2)	3.8 (2.5–4.9)	0.215	3.8 (2.8–5.2)	3.8 (2.5–4.5)	0.469
ICGR 15 (%)	17.9 (7.4–77.4)	18.7 (4.6–50.5)	0.734	17.65 (7.4–40.0)	17.55 (4.6–40.0)	0.561
AFP (ng/ml)	11.6 (0.5–3405)	27.6 (0.5–36572)	0.176	13.95 (0.5–3405)	22.9 (0.5–513)	0.635
Child–Pugh grade			0.665			0.556
A	41 (95.3%)	69 (90.8%)		37 (97.4%)	36 (94.7%)	
B	2 (4.7%)	7 (9.2%)		1 (2.6%)	2 (5.3%)	
Hepatic resection			0.322			0.373
Hr0	20 (46.5%)	49 (64.5%)		18 (47.4%)	23 (60.5%)	
HrS	13 (30.2%)	18 (23.7%)		12 (31.6%)	9 (23.7%)	
Hr1	3 (7.0%)	4 (5.3%)		2 (5.3%)	3 (7.9%)	
Hr2	7 (16.3%)	5 (6.6%)		6 (15.8%)	2 (5.3%)	
Hr3	0 (0%)	0 (0%)		0 (0%)	0 (0%)	
Operation time (min)	260 (128–623)	242 (90–580)	0.0514	257 (128–623)	247.5 (90–580)	0.18
Blood loss (ml)	200 (20–1900)	225 (10–960)	0.996	210 (20–1900)	210 (10–960)	0.803
Postoperative complications			0.933			0.798
IIIa	4	6		2	2	
IIIb	1	1		1	1	
IVa	1	1		1	0	
Stage			0.315			0.293
I	14 (32.6%)	19 (25.0%)		13 (34.2%)	9 (23.7%)	
II	18 (41.9%)	44 (57.9%)		15 (39.5%)	23 (60.5%)	
III	9 (20.9%)	12 (15.8%)		9 (23.7%)	6 (15.8%)	
IV-A	2 (4.7%)	1 (1.3%)		1 (2.6%)	0 (0.0%)	
Single tumor	28 (65.1%)	57 (75.0%)	0.252	25 (65.8%)	29 (76.3%)	0.312
Tumor size			0.712			0.589
≥3 cm	15 (34.9%)	24 (31.6%)		10 (26.3%)	8 (21.1%)	
<3 cm	28 (65.1%)	52 (68.4%)		28 (73.7%)	30 (78.9%)	
Vascular invasion	4 (9.3%)	3 (3.9%)	0.233	3 (7.9%)	0 (0.0%)	0.239

Continuous variables expressed as median (range)

Hepatic resection and stage were according to General Rules for the Clinical and pathological Study of Primary Liver Cancer, by Liver cancer Study Group of Japan, 5th edition, Kanehara Co., Ltd

Hr0: limited resection, HrS: segmentectomy, Hr1: sectionectomy, Hr2: hemihepatectomy, Hr3: more than hemihepatectomy

T-Bil total bilirubin, *PS* performance status, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ICGR 15* indocyanine green retention rate at 15 min, *AFP* alpha-fetoprotein,

did not receive IFN therapy was 3.8 (1.2–6.9) and 3.5 (1.3–6.8) years, respectively. In the matched study groups, the 3- and 5-year overall survival rates of patients who received peg-IFN therapy after hepatic resection were significantly higher than those of patients who did not receive IFN therapy ($P = 0.00135$) (Fig. 1c). However, there was no significant difference in disease-free survival between the two matched groups ($P = 0.886$) (Fig. 1d).

In the matched 38 patients of the peg-IFN group, peg-IFN therapy was initiated at a median of 4.3 (0.9–9.6) months after hepatic resection. Thirty-one of 38 HCC patients began peg-IFN therapy within 6 months after hepatectomy. Seven patients required more than 6 months to commence peg-IFN therapy. Two patients required a longer time to recover platelet counts of more than 70,000/μl. Five patients required a longer time to decide to receive peg-IFN therapy. Sixteen (42.1%) of the matched 38 patients who received peg-IFN therapy after hepatectomy attained SVR. Among 16 patients who attained SVR, 10 patients received full-dose peg-IFN therapy without dose reduction, whereas 6 patients received a reduced dose of peg-IFN and/or RBV until completion of treatment. Nine patients discontinued peg-IFN therapy because of adverse events such as thrombocytopenia and neutropenia ($n = 2$), skin eruption ($n = 1$), depression ($n = 2$), and severe malaise ($n = 4$). Three patients discontinued peg-IFN therapy because of HCC recurrence. Adherence to peg-IFN therapy was 68.4% in this study. No life-threatening adverse events were observed, and none of the total 15 deaths in both sets of matched patients were related to the IFN treatment or to surgical procedures. The 3- and 5-year overall survival rates of patients ($n = 16$) who attained SVR after peg-IFN therapy were 100% and 100%, respectively; those of patients who did not attain SVR ($n = 22$) were 100 and 85.7%, respectively; and those of patients who did not receive IFN therapy were 76.6 and 50.6%, respectively. There was a statistically significant difference in overall survival among the three groups ($P = 0.005$) (Fig. 2a). However, there was no statistically significant difference in disease-free survival among the three groups ($P = 0.90$) (Fig. 2b).

Table 2 presents the patterns of cancer recurrence and the treatment details of the recurrences in both groups. Twenty-one (55.3%) of the patients who received peg-IFN therapy after hepatic resection and 17 (44.7%) of the patients who did not receive IFN therapy had HCC recurrences after hepatic resection. Regarding the pattern of recurrence, the proportion of patients who had multiple

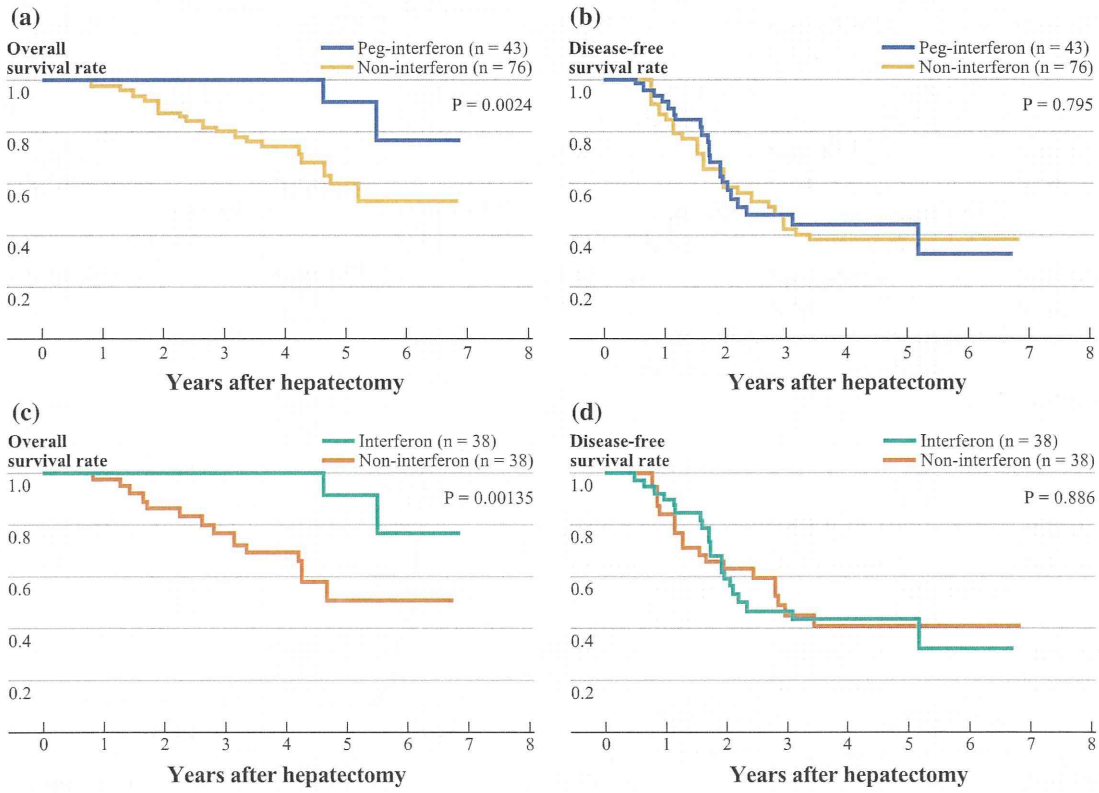

FIG. 1 Overall survival (**a**) and disease-free survival (**b**) of the entire study population of 175 patients with hepatitis C-related HCC with respect to IFN therapy after hepatic resection. Overall survival (**c**) and disease-free (**d**) survival of the matched study population of 76 patients with hepatitis C-related HCC with respect to IFN therapy after hepatic resection

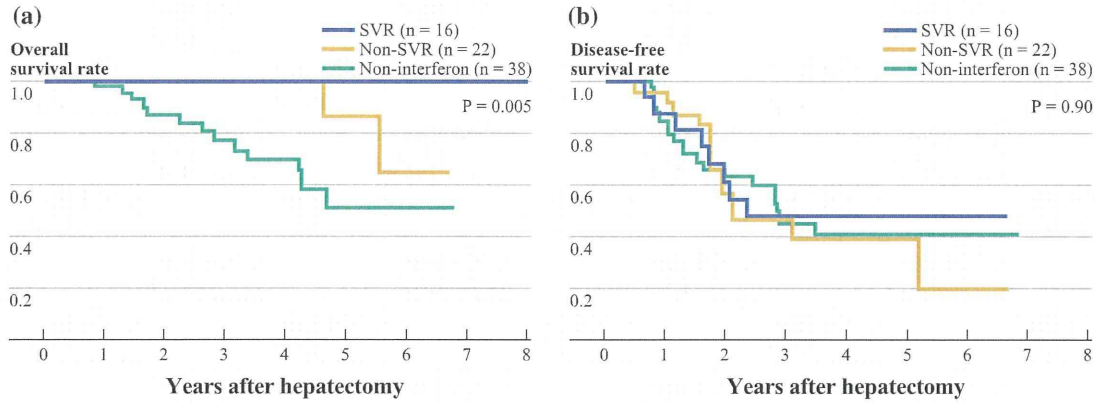

FIG. 2 Overall survival and disease-free survival of patients with hepatitis C-related HCC with respect to SVR after IFN therapy

intrahepatic recurrences (more than four nodules) was significantly lower in the peg-IFN group than in the non-IFN group ($P = 0.0047$). The proportion of patients in whom surgery or RFA was selected for treatment was significantly higher in the peg-IFN group than in the non-IFN group ($P = 0.0346$). Furthermore, regarding re-recurrence of HCC after treatment of the first-recurrent HCC, the 1-year disease-free survival rates of patients after treatment of the first-recurrent HCC was 48.5% in patients ($n = 21$) who received peg-IFN therapy and 12.5% in patients ($n = 17$) who did not receive IFN therapy. There was a statistically significant difference in disease-free survival between the two groups ($P = 0.0012$) (Fig. 3).

A comparison of results of the preoperative liver function test with those of postoperative 1-year liver function tests is presented in Table 3. In patients who received peg-IFN therapy, total bilirubin levels 1 year after surgery were significantly decreased compared with preoperative total bilirubin levels ($P = 0.018$), whereas in patients who did not receive IFN therapy, the total bilirubin level at 1 year after surgery was similar to the total bilirubin level before surgery ($P = 0.107$).

TABLE 2 Recurrence and treatments for recurrence after hepatic resection

	Peg-IFN (+) ($n = 38$)	IFN (−) ($n = 38$)	*P* value
HCC recurrence[a]: yes	21 (55.3%)	17 (44.7%)	0.359
Pattern of recurrence[b]			0.0047
Intrahepatic (single)	9 (42.9%)	8 (47.1%)	
Intrahepatic (2–3)	10 (47.6%)	1 (5.9%)	
Intrahepatic (multiple)	2 (9.5%)	8 (47.1%)	
Main modalities[b]			0.0346
Repeat hepatectomy	8 (38.1%)	2 (11.8%)	
RFA	8 (38.1%)	4 (23.5%)	
TACE	5 (23.8%)	11 (64.7%)	

peg-IFN pegylated interferon, *RFA* radiofrequency ablation, *TACE* transcatheter arterial chemoembolization

[a] Data expressed as number of patients (percentage of total patients)

[b] Data expressed as number of patients (percentage of patients who had a recurrence)

DISCUSSION

Our results revealed that peg-IFN therapy after hepatic resection improved the outcomes of HCV patients, although the interval of disease-free survival was not prolonged. Peg-IFN therapy after hepatectomy improved hepatic reserve function and suppressed multiple HCC recurrences (more than four nodules). Furthermore, re-recurrence after treatment of first-recurrent HCC after hepatic resection was significantly suppressed in the peg-IFN group compared with that in the non-IFN group. IFN has been reported to exert antitumor effects. IFN increases natural killer cell activity and exhibits antiangiogenic properties.[35,36] IFN has also been reported to be effective in eradicating HCV RNA

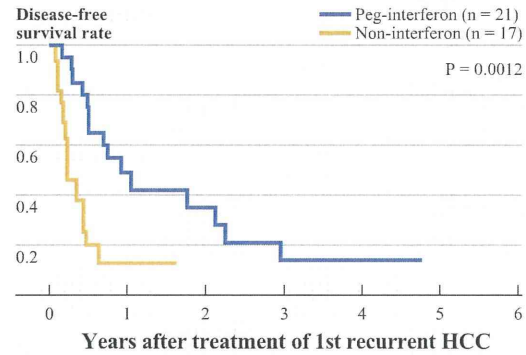

FIG. 3 Comparison of disease-free survival rate after treatment of first-recurrent HCC in patients who received peg-IFN therapy or in those who did not receive IFN therapy

TABLE 3 Comparison of preoperative liver function with 1-year liver function after hepatic resection

	Peg-IFN (+)		*P* value	IFN (−)		*P* value
	Preoperative	1 Year after surgery		Preoperative	1 Year after surgery	
T-Bil (mg/dl)	0.82 ± 0.29	0.71 ± 0.26	0.0189	0.81 ± 0.32	0.92 ± 0.35	0.107
AST (IU/l)	50.1 ± 24.1	45.8 ± 23.5	0.310	42.1 ± 18.9	56.1 ± 26.7	0.0110
ALT (IU/l)	51.3 ± 28.6	36.4 ± 22.8	0.00809	40.3 ± 24.3	49.7 ± 25.8	0.0918
Albumin (g/dl)	3.89 ± 0.80	3.99 ± 0.71	0.251	3.73 ± 0.45	3.75 ± 0.44	0.807

peg-IFN pegylated interferon, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

from serum and hepatic tissue, thereby preventing deterioration of liver function in patients with HCV infection.[37] IFN prevents worsening of compensated cirrhosis.[18,37] Our results are compatible with those reported in those studies. In the peg-IFN group, most patients with HCC recurrence could undergo curative treatments such as repeat hepatectomy or RFA as a recurrence treatment, because the number of recurrent tumors was usually limited to three. IFN therapy appears to increase survival not only by improving residual liver function and increasing the possibility of radical treatment of recurrences but also by suppressing re-recurrence after the first recurrence of HCC.

The current study also revealed that the overall survival of patients with SVR was significantly better than that of patients without SVR. This result suggests that IFN prolongs the outcomes of patients with HCC after hepatic resection by causing remission of active hepatitis and eradication of HCV RNA in patients who attained SVR after hepatic resection.

In this study, to clarify the impact of peg-IFN therapy on outcomes of HCV-related HCC after hepatic resection, patients who received IFNs such as IFN-α or IFN-β were excluded. RCTs investigating adjuvant effects of IFN after resection or ablation of HCC were performed using IFN-α. Few studies have investigated the effects of peg-IFN plus RBV combination therapy on survival and recurrence after curative resection of HCC. Combination therapy with peg-IFN and RBV has recently been developed, and peg-IFN therapy has resulted in significantly higher SVR rates and better tolerability than treatment with IFN-α.[21,23] In our study, incidence of SVR after hepatic resection was 42.1%, which was higher than that in previous studies that reported an SVR rate of 0–10%.[12–14] The compliance of patients to peg-IFN therapy observed in the present study (68.4%) was higher than that reported elsewhere (approximately 40%).[14] This enhanced efficacy of the peg-IFN formulations might contribute to the prolonged survival of HCC patients after hepatic resection.

In this study, HCC patients who received peg-IFN therapy within 9 months after surgery were enrolled, and HCC patients who experienced recurrence of HCC within 9 months after hepatic resection were excluded from the non-IFN group, because these patients could lose the opportunity to receive IFN therapy for HCC recurrence on being assigned to the peg-IFN therapy group.

Before matching by using the propensity score, the clinical characteristics of the entire study population that can strongly influence outcomes differed significantly between the peg-IFN group and non-IFN group. The proportion of older patients was higher in the non-IFN group than in the peg-IFN group, whereas the proportion of patients who had longer operation times tended to be lower in the non-IFN group than in the peg-IFN group. To overcome bias due to the different distribution of the severity of liver function impairment between the two groups, a one-to-one match was created using propensity score analysis. After matching by propensity score, prognostic variables were appropriately handled, and there was no significant difference in prognostic factors between the two matched groups. This study had a limitation related to the small sample size after propensity score matching. To overcome this, further examination with larger sample sizes is necessary, and the potential efficacy of peg-IFN therapy must be validated in larger prospective RCTs.

CONCLUSIONS

Several previous RCTs investigating the effects of IFN on survival and tumor recurrence after hepatic resection were inconclusive. However, in the current study, peg-IFN therapy following hepatic resection improved the survival rates of hepatectomized patients with HCV-related HCC. The results of this study suggest that peg-IFN therapy is effective as an adjuvant chemopreventive agent after hepatic resection in patients with HCV-related HCC.

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