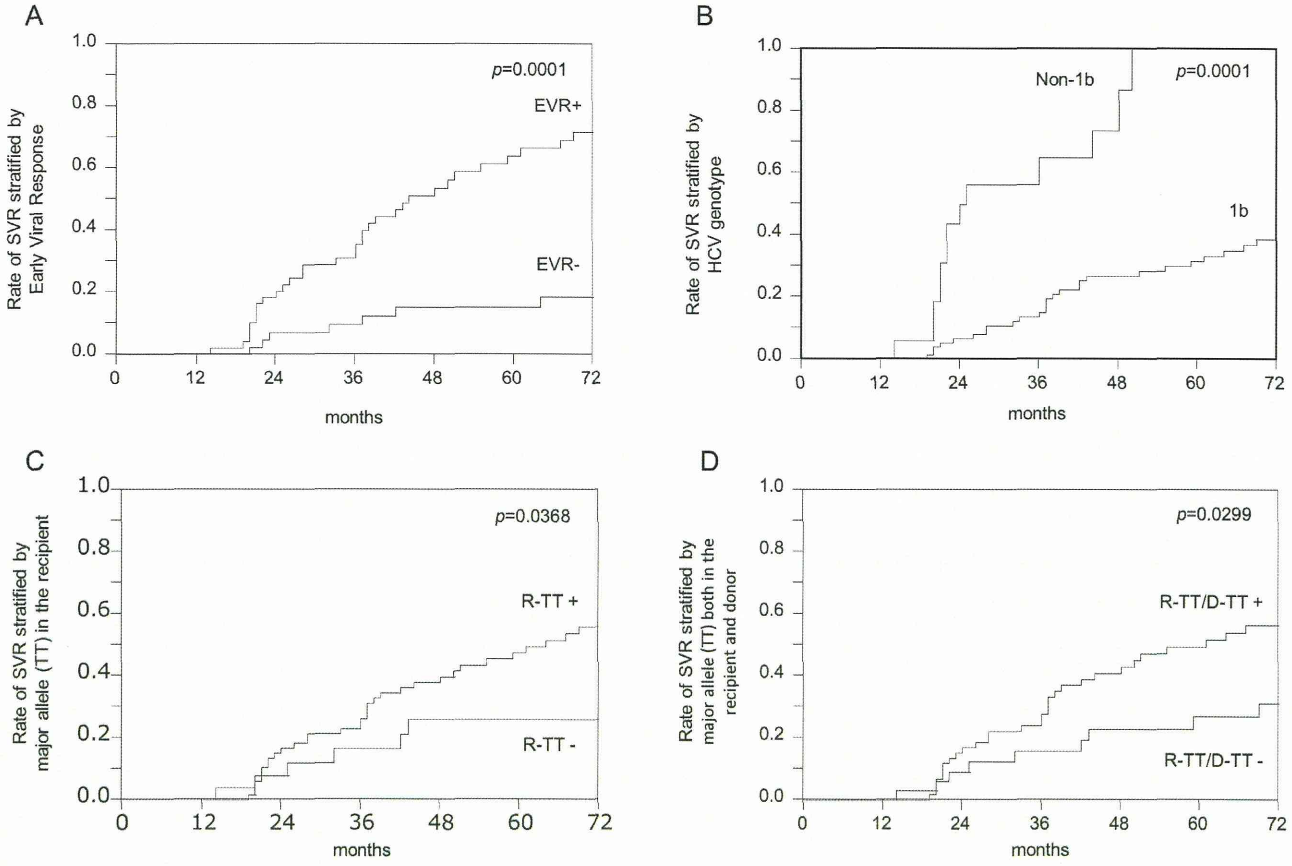

Figure 3. Cumulative rates of SVR based on the Kaplan-Meier method. A. Stratified by EVR, B. HCV genotype, C. major allele (TT) in the recipient, and D. major allele (TT) both in the recipient and donor. Abbreviations: SVR, sustained viral response; EVR, Early viral response; R-TT, major allele (TT) in the recipient; D-TT, major allele (TT) in the donor.
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($p = 0.0219$ and $p = 0.0212$ for all recipients and for recipients with genotype 1b HCV, respectively). However, difference of SVR according to the donor IL28B SNPs did not reach statistical significance ($p = 0.3029$ and $p = 0.2279$ for all donors and for donors whose recipients with genotype 1b, respectively).

The combinations of recipient and donor IL28B polymorphisms and corresponding rates of virologic responses are summarized in Figures 2A–2D. Although combinations of recipients and grafts obtained from donors both carrying the major homozygous allele presented with tendency of higher rates of virologic eradication demonstrated as ETR (Figure 2C) or SVR (Figure 2D), especially in the case of HCV RNA genotype 1b, this synergistic tendency remained unclear or limited when observation was extended to on-treatment virologic response at an earlier stage evaluated by temporal clearance or EVR (Figures 2A and 2B). For example, rates of EVR between recipient and donor pairs carrying both a major homozygous allele and minor allele did not differ significantly ($p = 0.9416$), and the advantage remained unclear even when limited to the HCV RNA genotype 1b ($p = 0.5804$) (Figure 2A). Similarly, the presence of a minor allele either in the recipient or the donor did not significantly affect temporal viral clearance (Figure 2B).

Impact of IL28B polymorphism among other factors

Impact of various clinical factors on sensitivity to interferon treatment in the present study was assessed by SVR rates (Table 1). Compatible with previous studies, univariate analysis revealed that HCV genotype 1b and presence of EVR were significant factors affecting outcome. As for IL28B polymorphisms, a major allele homozygote in the recipient, and a major allele homozygote both in the recipient and donor presented with a statistically significant impact ($p = 0.0368$, and 0.0299, respectively). A major allele homozygote in the donor side alone did not have strong impact (0.3136; Table 1). The above four factors significantly impacted SVR in univariate analysis are summarized in Figure 3.

To elucidate the magnitude of the IL28B polymorphism, a multivariate study was conducted including all clinical variables from Table 1. To incorporate the nature of our treatment protocol without a defined period of interferon treatment, multivariate analysis was performed using the Cox proportional-hazards model. The study revealed the genotype of HCV and EVR, but not the IL28B polymorphism of either recipient or donor, to be independent factors to achieve SVR. Recipients re-infected with HCV genotype 1b presented with a significantly poorer chance of SVR (Hazard ratio 0.277, 95% confidence interval 0.132–582, $p = 0.0007$). On the other hand, once EVR was observed, recipients demonstrated significantly better opportunity for SVR (Hazard ratio 4.426, 95% confidence interval 1.958–10.007, $p = 0.0004$). Cumulative rates of SVR within the genotype 1b or non-1b population stratified by the presence of EVR are presented in Figure 4. Conversely, background factors of recipients presenting EVR or not so depending on HCV RNA genotypes

Table 1. Sustained viral response in patients with combined treatment and clinical factors.

Factors		No.	%SVR at 5 y	p
R-age	=<60	72	48	
	>60	24	26	0.5221
R-sex	Male	70	44	
	Female	26	34	0.5895
MELD	=<15	52	42	
	>15	44	40	0.9585
HIV	Positive	3	67	
	Negative	93	40	0.0729
HCC	Positive	60	41	
	Negative	36	48	0.7344
Genotype	1b	79	32	
	Non-1b	17	100	<0.0001
Graft size	<50%	53	46	
%R-SLV	>=50%	43	37	0.8251
HCV-RNA	=<5.6	48	45	
titer	>5.6	48	38	0.2999
EVR	Yes	49	64	
	No	47	15	<0.0001
ACR	Yes	20	49	
	No	76	39	0.3844
D-age	=<40	61	39	
	>40	35	46	0.1101
D-sex	Male	62	33	
	Female	34	59	0.1155
CyA	Yes	58	51	
	No	38	27	0.0683
R-IL28B	TT	69	47	
	TG/GG	27	26	0.0368
D-IL28B	TT	77	44	
	TG/GG	19	31	0.3136
R-TT/D-TT	Yes	61	49	
	No	35	27	0.0299
R-TT/D-TG+GG	Yes	8	31	
	No	88	42	0.7276
R-TG+GG/D-TT	Yes	16	22	
	No	80	45	0.0962
R-TG+GG/D-TG+GG	Yes	11	31	
	No	85	43	0.3386

Abbreviations: No., number of patients; %SVR, rate of recipients achieving sustained viral response; R-age, age of the recipient at the time of transplantation; R-sex, sex of the recipient; MELD, Model for end-stage liver disease score; HIV, human immune deficiency virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; %R-SLV, percentage of graft size to recipient's standard liver volume; HCV-RNA, hepatitis C viral ribonucleic acid; EVR, early viral response; ACR, acute cellular rejection; D-age, age of the donor at the time of transplantation; D-sex, sex of the donor; CyA, cyclosporine A; R-IL28B, recipient's IL28B polymorphism (rs8099917); D-IL28B, donor's IL28B polymorphism (rs8099917); R-TT/D-TT, Both recipient and donor carrying major allele (TT); R-TT/D-TG+GG, recipient carrying major allele (TT) but donor carrying minor allele (TG or GG); R-TG+GG/D-TT, recipient carrying minor allele (TG or GG) but donor carrying major allele (TT); R-TG+GG/D-TG+GG, Both recipient and donor carrying minor allele (TG or GG).

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were evaluated (Table 2). Among various clinical factors, IL28B polymorphisms, either that of the recipient or the donor, or both, was not prevalent in relation to EVR, especially in the genotype 1b population.

Overall, IL28B polymorphisms had a relative, not independent, impact. On-treatment virologic response to interferon and RBV-based treatment represented by EVR remain the most significant factor predicting SVR, even among recipients with HCV genotype 1b.

Discussion

Treatment of HCV re-infection by interferon and ribavirin after liver transplantation has remained a challenge with inferior outcomes compared to the non-transplantation population due to immunosuppression, and low tolerability. Recurrence and persistence of HCV-infection remain the most common cause of post-transplant graft loss and mortality. Identifying factors affecting the outcomes, including the response to treatment, therefore, continues to be a subject of keen interest. This study presents observations that may be potentially important in light of advancements involving recent genetic discoveries regarding IL28B polymorphisms.

This is the largest study to date, 96 LDLT cases, evaluating the impact of IL28B polymorphisms in both donor and recipient, in accordance with the on-treatment response, on the outcome. Also, the series is the first to analyze the magnitude of the polymorphism under a preemptive treatment approach [17,18] after LDLT. The study is limited to rs8099917 based on previous studies of IL28B SNPs in the Japanese population, and therefore, impact of rs12979860 awaits further similar study in the West.

Several predictive factors for interferon and RBV sensitivity in the non-transplant population were recently identified. Virologically, HCV-genotype, and HCV RNA mutations in the core and NS5A regions are recognized as important factors [24,25]. As for host factors, by genome-wide association study coming from three independent studies, IL28B polymorphisms have been identified as significant factors affecting virologic clearance [19–21]. The mechanism underlying the influence of IL28B polymorphisms on the response to interferon and RBV therapy is, however, yet to be determined. Current understanding is that the product of the IL28B gene is interferon lambda-3, which belongs to the type III interferon family that induces interferon-stimulated genes. Favorable IL28B polymorphisms are associated with decreased levels of intrahepatic interferon-stimulated genes, offering a favorable environment for virologic clearance under interferon and RBV treatment [26–28]. Whether or not this mechanism is applicable to the liver transplant setting, in which a liver allograft is infected by HCV under immunosuppression, remains to be studied. There is little evidence to speculate otherwise at this point.

Fukuhara and colleagues [22] first reported the impact of IL28B polymorphisms on the outcome of LDLT. In their study, IL28B polymorphisms were studied in 67 HCV infected recipients and 41 living liver donors. Interestingly, they reported that SVR achievement was significantly associated with IL28B polymorphisms of both the recipient and donor. When both were major-allele homozygotes, the SVR rate was 56%. Whereas when either the recipient or the donor presented with a minor heterozygote or homozygote allele, SVR rate dropped to 10%, and further, when both the recipient and donor presented with a minor heterozygote or homozygote allele, none achieved SVR. Kawaoka and colleagues [23] conducted a similar study involving 20 LDLT recipient and donor pairs. They reported that major-allele homozygotes in both the recipient and donor resulted in an

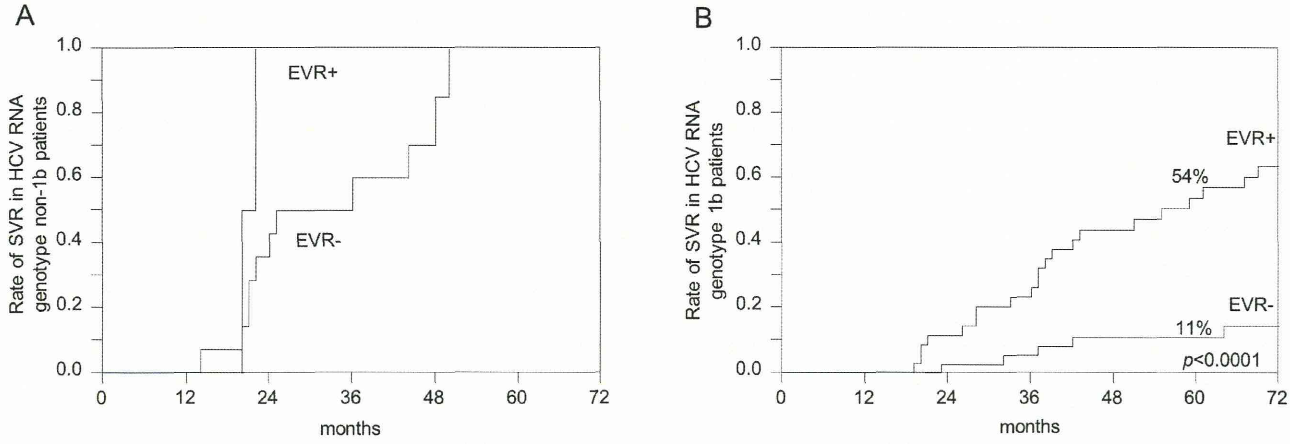

Figure 4. Cumulative rates of SVR stratified by EVR using the Kaplan-Meier method. A. HCV RNA genotype non-1b, B. HCV RNA genotype 1b. Abbreviations: SVR, sustained viral response; EVR, Early viral response.
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SVR rate of 81%. Although the number of cases where rather small, multivariate analysis including adherence to RBV therapy was performed, revealing that major-allele homozygotes in both the recipient and donor as the only independent and dominant determinant of SVR with and an odds ratio of 15.

Although logistic and technical difficulties remain in sampling and analyzing IL28B gene of the donors, comparable studies have been performed in the deceased donor setting [29–34]. The impact of IL28B polymorphisms has also become recognized in deceased donor liver transplantation for HCV. It was suggested that, patients requiring liver transplantation due to end-stage chronic HCV appeared to be selected toward the adverse genotypes [31], and the polymorphism seems to influence the degree of graft inflammation at biochemical and histologic levels following transplantation [29,32]. It has also become evident that, while there seems to be little doubt that IL28B polymorphisms markedly affect the response to interferon and RBV treatment, whether the donor or recipient, or the combination of both, should be considered paramount differ among studies. Lange and colleagues provide evidence that the donor's rather than the recipient's IL28B genetic background has a dominant impact on the virologic response [31], while Cotpo-Llerena et al. [34] report that the recipient's genetic background plays a major role. On the other hand, a recent study by Duarte-Rojo and colleagues [30] used multivariate analysis to demonstrate that the combination of both is the most influential. A German study [32] provided no data on the potential role of donor IL28B polymorphisms. The numbers of subjects in these studies remain small in comparison with the size of patients involved in analysis of non-transplant cases. Data on previously reported important clinical factors other than IL28B polymorphisms are not readily available for evaluation; much less an analysis by multivariate analysis to weigh the impact in a more reliable context. Clearly, further studies are required with an inclusion of a broader range of clinical data.

In our study, we included factors previously reported to influence the outcomes of the virologic response against interferon and RBV therapy in the analysis. This includes age and sex of both the donor and recipient, preoperative viral load, immunosuppression, and other factors as well as the on-treatment results represented by EVR (Table 1). A recent report by Thompson and colleagues suggests that on-treatment virologic response may have strong predictive power regardless of the IL28B type [35]. We used multivariate analysis that included all of these factors, and found that IL28B polymorphism is an influential but not determinant factor for SVR. Rather, in our series with a preemptive treatment approach, we demonstrated that on-treatment response was the key factor for predicting SVR.

Our study has three major weaknesses. First, although clinical virologic response was followed up and recorded in a prospective manner, IL28B polymorphisms were recently determined, making our study a retrospective case series with diverse sources of DNA. DNA samples for analysis were collected either from peripheral blood mononuclear cells or from formalin-fixed paraffin-embedded samples based on availability. A prospective study with a fixed DNA sampling protocol is required. Second, the nucleotide sequences of the core and non-structural 5A regions, another recently suggested important factor [36], have not been investigated in concert with IL28B polymorphisms. In fact, few studies to date have performed a combined analysis of both IL28B polymorphisms and HCV RNA nucleotide sequences, most likely due to the additional logistic burden. Fukuhara and colleagues [22] reported the synergistic value of combining findings from IL28B polymorphisms and HCV RNA nucleotide sequences in predicting the treatment response. This aspect should also be considered in future studies. Third, the study lacks histologic data. In our series, protocol biopsy was not performed. Hepatic venous gradient to evaluate the degree of liver fibrosis was also not routinely performed. This is due in part to our preemptive treatment strategy. Eurich and colleagues [32] have presented interesting outcomes regarding the progression of the histologic response in their deceased donor series. Comparable analysis in the living donor setting in the future may be valuable.

Finally, in the current study, direct antiviral agents in combination with peg-IFN and ribavirin were not used. Although the efficacy of the earlier generation of direct antiviral agents has become recognized in the non-transplant population, drastically altering standard treatment [37–39], its safety and effectiveness under routine use in the transplant population await future confirmation. Development in this aspect, however, is in rapid progression. Current recognition is that new-age anti-HCV treatment incorporating advanced direct antiviral agents will radically alter the outcome [40]. Further accumulation of data in combination with IL28B and the development of additional treatment options may be beneficial.

Table 2. Early viral response in patients with combined treatment and clinical factors.

Factors		HCV RNA Genotype Non-1b EVR No	Yes	p	1b EVR No	Yes	p
R-age	=<60	2	11		31	28	
	>60	1	3	1.0000	13	7	0.4368
R-sex	Male	2	10		33	25	
	Female	1	4	1.0000	11	10	0.8001
MELD	=<15	3	5		26	18	
	>15	0	9	0.0824	18	17	0.6488
HIV	Positive	0	3		0	0	
	Negative	3	11	1.0000	44	35	NA
HCC	Positive	2	5		28	25	
	Negative	1	9	0.5368	16	10	0.4826
Graft size	<50%	1	7		26	19	
%R-SLV	>=50%	2	7	1.0000	18	16	0.8194
HCV-RNA titer	=<5.6	3	8		24	13	
	>5.6	0	6	0.5147	20	22	0.1735
ACR	Yes	0	2		7	11	
	No	3	12	1.0000	37	24	0.1149
D-age	=<40	1	7		28	25	
	>40	2	7	1.0000	16	10	0.4826
D-sex	Male	2	8		27	25	
	Female	1	6	0.6704	17	10	0.4744
CyA	Yes	0	1		19	18	
	No	3	13	1.0000	25	17	0.5029
R-IL28B	TT	3	10		29	27	
	TG/GG	0	4	0.5412	15	8	0.3251
D-IL28B	TT	1	11		36	29	
	TG/GG	2	3	0.1912	8	6	1.0000
R-TT/D-TT	Yes	1	10		26	24	
	No	2	4	0.5147	18	11	0.4826
R-TT/D-TG+GG	Yes	2	0		3	3	
	No	1	14	0.0221	41	32	1.0000
R-TG+GG/D-TT	Yes	0	1		10	5	
	No	3	13	1.0000	34	30	0.3983
R-TG+GG/D-TG+GG	Yes	0	3		5	3	
	No	3	11	1.0000	39	32	1.0000

Abbreviations: HCV-RNA, hepatitis C viral ribonucleic acid; EVR, early viral response; R-age, age of the recipient at the time of transplantation; R-sex, sex of the recipient; MELD, Model for end-stage liver disease score; HIV, human immune deficiency virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; %R-SLV, percentage of graft size to recipient's standard liver volume; ACR, acute cellular rejection; D-age, age of the donor at the time of transplantation; D-sex, sex of the donor; CyA, cyclosporine A; R-IL28B, recipient's IL28B polymorphism (rs8099917); D-IL28B, donor's IL28B polymorphism (rs8099917); R-TT/D-TT, Both recipient and donor carrying major allele (TT); R-TT/D-TG+GG, recipient carrying major allele (TT) but donor carrying minor allele (TG or GG); R-TG+GG/D-TT, recipient carrying minor allele (TG or GG) but donor carrying major allele (TT); R-TG+GG/D-TG+GG, Both recipient and donor carrying minor allele (TG or GG).
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Conclusions

In contrast to previous reports, when virologic response to treatment was incorporated into analysis, the impact of IL28B polymorphism on achieving SVR remained relative in our living donor liver transplantation series under a preemptive interferon and RBV-based treatment approach. HCV genotype 1b and on-treatment response represented by EVR were both significant and independent factors. Caution should be used when incorporating the IL28B polymorphism into the treatment strategy of HCV reinfection following liver transplantation in an absolute manner, such as to the donor selection or graft allocation, however, until the mechanism of its effect is elucidated and well-designed future studies have confirmed its true nature.

Author Contributions

Conceived and designed the experiments: NH ST Y. Sugawara. Performed the experiments: JT TI JK TA Y. Sakamoto. Analyzed the data: KH TT NY. Contributed reagents/materials/analysis tools: NK. Wrote the paper: NH ST Y. Sugawara.

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REVIEW

Living-donor *vs* deceased-donor liver transplantation for patients with hepatocellular carcinoma

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Abstract

With the increasing prevalence of living-donor liver transplantation (LDLT) for patients with hepatocellular carcinoma (HCC), some authors have reported a potential increase in the HCC recurrence rates among LDLT recipients compared to deceased-donor liver transplantation (DDLT) recipients. The aim of this review is to encompass current opinions and clinical reports regarding differences in the outcome, especially the recurrence of HCC, between LDLT and DDLT. While some studies report impaired recurrence - free survival and increased recurrence rates among LDLT recipients, others, including large database studies, report comparable recurrence - free survival and recurrence rates between LDLT and DDLT. Studies supporting the increased recurrence in LDLT have linked graft regeneration to tumor progression, but we found no association between graft regeneration/initial graft volume and tumor recurrence among our 125 consecutive LDLTs for HCC cases. In the absence of a prospective study regarding the use of LDLT *vs* DDLT for HCC patients, there is no evidence to support the higher HCC recurrence after LDLT than DDLT, and LDLT remains a reasonable treatment option for HCC patients with cirrhosis.

Key words: Deceased donor liver transplantation; Hepatocellular carcinoma; Living donors; Living-donor liver transplantation; Recurrence

Core tip: The current opinions and clinical reports regarding differences in the recurrence of hepatocellular carcinoma (HCC) between living donor liver transplantation (LDLT) and deceased donor liver transplantation (DDLT) were reviewed. In the absence of a prospective study regarding the use of LDLT *vs* DDLT for HCC patients, only with some retrospective studies with conflicting results, there is no evidence to support the higher HCC recurrence after LDLT than DDLT, and LDLT remains a reasonable treatment option for HCC patients with cirrhosis.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the 7th most common cancer overall and the 3rd most common cause of cancer-related death worldwide[1,2]. Since the landmark report of the Milan criteria by Mazzaferro *et al*[3], which demonstrated comparable outcomes of patients with HCC having a single tumor smaller than 5 cm in diameter or up to 3 tumors smaller than 3 cm in diameter with no vascular invasion or extra-hepatic disease determined by preoperative imaging studies, deceased - donor liver transplantation (DDLT) has become an established treatment for cirrhotic patients with HCC[4,5]. Similarly, in Asian countries where living-donor liver transplantation (LDLT) comprises the majority of liver transplantation procedures, LDLT has become an established treatment

Table 1 Studies comparing living - donor liver transplantation and deceased - donor liver transplantation for hepatocellular carcinoma

Ref.	Country	Year	Study period	Type of LT	Case number	Recurrence - free survival			*P*	% Recurrence rate	*P*	Criteria used	% Outside Milan	Difference in tumor characteristics	Median follow-up period (mo)
						1-yr	3-yr	5-yr							
Impaired results in LDLT															
Park *et al*[10]	South Korea	2014	1999-2010	LDLT	166	89		81	0.045	19	0.045	UCSF	NA	none	35
				DDLT	50	96		94		6					
Vakili *et al*[13]	United States	2009	1999-2007	LDLT	28					29	< 0.05	UNOS	25	none	41
				DDLT	65					12					
Kulik *et al*[12]	United States Multi-center	2012	1998-2010	LDLT	100	80	66	56	0.05	38	0.0004	UNOS	59	More aggressive in LDLT	60
				DDLT	97	90	81	73		11			30		
Lo *et al*[14]	Hong Kong	2007	1995-2004	LDLT	43	93	71	71	0.029	29	0.029	UCSF	26	More aggressive in LDLT	33
				DDLT	17	100	100	100		0			29		
Comparable results															
Sandhu *et al*[15]	Canada	2013	1996-2009	LDLT	58	88	75	70	NS	17	NS	Toronto criteria	28	none	38
				DDLT	287	86	75	70		15			32		31
Bhangui *et al*[16]	France	2011	2000-2009	LDLT	36	100	89	88	NS	13	NS	UCSF	27	none	58
				DDLT	120	93	89	86		13			21		50
Li *et al*[36]	China	2010	2005-2009	LDLT	38	71	42		NS	50	NS	UCSF	79	none	25
				DDLT	101	76	41			55			68		
Di Sandro *et al*[35]	Italy	2009	2000-2007	LDLT	25		96	96	NS	4	NS	Milan	20	none	NA
				DDLT	154		91	89		11			31		
Sotiropoulos *et al*[20]	Germany	2007	1998-2006	LDLT	45	88	75		NS	12	NS	UCSF	44	none	NA
				DDLT	55		81			14					
Hwang *et al*[8]	South Korea Multi-center	2005	1992-2002	LDLT	237	83	80		NS	18	NS		27	none	26
				DDLT	75	88	82			16			29		45
Gondolesi *et al*[17]	United States	2004	1988-2002	LDLT	36	82	74		NS	19	NS	UNOS	53	none	15
				DDLT	165	90	83			19					

DDLT: Deceased - donor liver transplantation; HCC: Hepatocellular carcinoma; LDLT: Living - donor liver transplantation; LT: Liver transplantation; UCSF: University of California, San Francisco; UNOS: United Network for Organ Sharing; NA: Not applicable; NS: Not significant.

for HCC patients with end-stage liver disease[6,7]. LDLT is now considered a promising treatment for HCC patients in Western countries, not only to compensate for the shortage of donor organs but also to reduce the dropout rate on the waiting list[8].

With the accumulation of LDLTs for HCC patients, the impact of LDLT on recipient outcome compared with DDLT, especially the recurrence of HCC after liver transplantation, has become an important topic of debate[9]. The aim of this review was to encompass the current opinions and clinical reports regarding the differences in outcome, especially the recurrence of HCC, between LDLT and whole liver DDLT.

STUDIES COMPARING LDLT AND DDLT FOR HCC PATIENTS

Studies comparing LDLT and DDLT for HCC patients are summarized in Table 1. All DDLTs reviewed here were done with the whole liver graft.

Studies reporting a poorer outcome in the LDLT setting

Park *et al*[10] recently reported poorer recurrence-free survival among 166 LDLT recipients (81% at 5 years) compared to 50 DDLT recipients (94% at 5 years; $P = 0.045$). The noteworthy finding of this study was that the smaller the LDLT graft, the poorer the recurrence - free survival. Based on this finding, Park *et al*[10] suggested that the physiology of the small graft may stimulate tumor recurrence.

The results of the A2ALL cohort in United States also demonstrated an impaired outcome in LDLT recipients. In their initial report[11], they found a higher rate of recurrence within 3 years in LDLT than in DDLT (29% *vs* 0%, $P = 0.002$), but there was a clear tendency toward more aggressive tumor characteristics in the LDLT group. The same group recently published an updated report[12], in which HCC recurrence remained significantly different between LDLT and DDLT after adjustment for tumor characteristics. They concluded that the higher recurrence observed after LDLT was likely due to differences in the tumor characteristics, pretransplant HCC management, and waiting time.

Vakili *et al*[13] reporting the Lahey Clinic experience, demonstrated that the HCC recurrence rate of LDLT (29%) was significantly higher than that of DDLT (12%) ($P < 0.05$), but survival after LDLT was significantly better than that following DDLT for HCC during the same period ($P = 0.02$).

Lo *et al*[14] from Hong Kong also reported a significantly higher incidence of HCC recurrence, 29% in LDLT and 0% in DDLT ($P = 0.029$). While the tumor characteristics were comparable between groups, the authors speculated that LDLT as a salvage transplantation, microscopic vascular invasion, and liver regeneration led to the difference in the recurrence rate.

Studies reporting a comparable outcome

Sandhu and colleagues of the Toronto group[15] reported that LDLT and DDLT both provide similarly low recurrence rates and high survival rates. They compared the results of 58 LDLT cases with those of 287 DDLT cases having comparable tumor characteristics, in which the 1-, 3-, and 5-year recurrence-free survival rates were 88%, 75%, and 70%, and 86%, 75%, and 70%, respectively.

In a well-designed study by Bhangui *et al*[16], an intention-to-treat analysis was conducted with recurrence rate representing the primary endpoint, comparing 36 LDLT cases and 147 DDLT cases. The authors demonstrated that both LDLT and DDLT provided similar recurrence - free survival rates (88% *vs* 86% at 5 years) for patients with HCC. The dropout rate and waiting time were significantly lower in the LDLT group than in the DDLT group, and there was also a trend toward a longer time to recurrence in the LDLT group, which may guarantee additional advantages with LDLT.

The Mount Sinai group[17,18] reported comparable recurrence - free survival between LDLT ($n = 36$) and DDLT ($n = 165$; 74% *vs* 83% at 2 years, $P = 0.3$). When stratified by tumor size (5 cm diameter) and the existence of microvascular invasion, there was still no difference between groups.

Sotiropoulos and colleagues of Essen, Germany[19,20], also supported the comparable recurrence - free survival rates between LDLT and DDLT for HCC (75% *vs* 81% at 3 years).

Hwang *et al*[21] of South Korea performed a nationwide survey regarding this issue. Among 237 LDLTs and 75 DDLTs for HCC, the 1 - and 3 - year recurrence - free survival rates were 83% and 80%, and 88% and 82%, respectively, with no significant difference between them.

A comparison of outcomes after liver transplantation obtained from database studies revealed comparable patient survival rates between LDLT and DDLT. According to a report from the Japanese Liver Transplantation Society Registry[22], a total of 6097 LDLTs were performed in Japan by the end of 2010, and 1225 (32%) were indicated for HCC, which was the most common indication in adult patients. The 1-, 3-, 5-, and 10-year cumulative survival rates of LDLT for HCC were 85%, 74%, 69%, and 60%, respectively. Todo and colleagues[23] performed a detailed survey using the same database (up to the end of 2005), comprising 653 patients who had undergone LDLT for HCC in Japan. At 1, 3, and 5 years, overall patient survival was 83%, 73%, and 69%, and disease-free survival was 77%, 65%, and 61%, respectively. Based on preoperative imaging studies, 62% were within the Milan criteria and 38% were beyond the Milan criteria, with 5-year recurrence-free survival rates of 90% and 61%, respectively ($P < 0.001$). These findings do not differ much from those obtained in the DDLT database of the United States and Europe[24-27], and may validate the use of LDLT for HCC patients.

CURRENT OPINIONS REGARDING THE DIFFERENCE BETWEEN LDLT AND DDLT

A randomized clinical study would be best to settle the controversy regarding the use of LDLT *vs* DDLT for HCC patients, but this is indeed difficult, if not impossible, to realize given the complicated decision-making process involved in LDLT. No prospective study has been conducted to date.

The Toronto group[28] recently performed a meta-analysis on 12 retrospective studies comparing the recurrence rates and recurrence - free survival between LDLT and DDLT recipients. A total of 633 LDLTs and 1232 DDLTs were enrolled, and the study provided evidence of lower disease - free survival after LDLT compared with DDLT for HCC (HR = 1.59, 95%CI: 1.02-2.49; $P = 0.041$). In contrast, there was no difference in overall survival between LDLT and DDLT (HR = 0.97, 95%CI: 0.73-1.27; $P = 0.808$). As mentioned by the authors of the paper, however, all involved studies were retrospective, had a low data quality score with poor reporting of baseline patient characteristics and an inadequate statistical approach, and were heterogeneous in critical aspects such as indication criteria and basal tumor characteristics, which warrant further well-designed studies to determine whether differences in HCC recurrence are due to study biases or biologic differences.

A recent review article by experts[29] concluded as follows: Although there is no strong evidence to support the higher HCC recurrence rates in LDLT than DDLT, the higher recurrence rates in LDLT recipients reported by several authors cannot be ignored. Actually, there are critical differences among societies such as: (1) differences in the allocation system for DDLT and LDLT; (2) differences in the availability of deceased donors; (3) differences in the potential waiting time; and (4) the differences in regional and national organ transplant law. In addition to taking into account these differences, liver transplant candidates with HCC and their potential live donors should be informed following risks and benefits; the waiting time for DDLT may lead to the dropout due to HCC progression which could be avoided by the prompt LDLT, however, the prompt LDLT may mask the aggressive tumor characteristics which may lead to a higher HCC recurrence rates. Although the currently available literatures can provide a low evidence for the difference of HCC recurrence between DDLT and LDLT, the tumor characteristics and biology seem to significantly influence on the recurrence, while the graft type and waiting time are less likely important as a possible risk factor.

Table 2 Graft characteristics and hepatocellular carcinoma recurrence

	Patients with recurrence (*n* = 11)	Patients without recurrence (*n* = 114)	*P*
Regeneration rate at 3 mo (%)	90 ± 24	93 ± 34	0.732
Graft type: right/left	4/7	36/78	0.702
Initial graft volume ratio to standard liver volume (%)	46 ± 9	47 ± 9	0.842

POSTULATED THEORIES FOR DIFFERENCES BETWEEN LDLT AND DDLT

LDLT provides several advantages compared with DDLT, such as a shorter waiting time, good quality graft with normal liver function and shorter ischemic time, and pretransplant treatment optimization, which might contribute to improved survival in LDLT recipients. Some of these characteristics, on the other hand, may lead to a favorable milieu for tumor progression[9].

There are several hypotheses other than tumor characteristics to explain the inferior outcome of LDLT. One explanation for the higher recurrence rates in LDLT is fast-tracking patients into liver transplantation, the so - called fast-track effect[11,30]. Some patients with more biologically aggressive HCC might drop off the waiting list due to tumor progression beyond the criteria during the wait-time in the DDLT setting. In contrast, due to the shortened wait time for LDLT candidates, progression of HCC with an aggressive tumor biology might not be recognized during such a short wait-time. This scenario might account for the higher HCC recurrence in the LDLT setting.

Another hypothesized mechanism for the higher recurrence rates in LDLT is that growth factors and cytokines released during rapid regeneration of the partial grafts from living donors might contribute to tumor progression and recurrence[31-34]. A rapidly regenerating liver parenchyma and ischemic-reperfusion injury facilitated by a small-for-size graft in LDLT setting might be a more favorable environment for tumor progression and HCC recurrence.

Additionally, some authors[11,35,36] insist that the technique of LDLT per se foregoes the principles of oncologic surgery. During LDLT, the meticulous dissection and mobilization of the liver might increase the possibility of tumor capsule violation or tumor embolization through the hepatic veins, thus promoting tumor dissemination. Preserving the native vena cava and the bile duct/hepatic artery/portal vein in the hepatic hilum might increase the risk of leaving the residual tumors.

As opposed with the above-mentioned anecdotal explanations, the advanced tumor characteristics of LDLT recipients can reasonably explain the higher recurrence rate in the LDLT setting. Grafts from living donors are not limited by restrictions imposed by the organ allocation system, meaning that the relation of the graft and recipient is usually one-on-one. Consequently, selection criteria based on the tumor burden, such as the tumor size and number, can be considered relative on a case-by-case basis, taking into account the presence of risk factors for recurrence and the chance of survival, as well as the wishes of the donor[37]. Consequently, the majority of Asian transplant centers have adopted extended criteria beyond those of Milan or the University of California, San Francisco (UCSF)[38]. Based on some studies, differences in patient tumor characteristics between LDLT and DDLT remain a main reason for the higher recurrence rate in LDLT. Additionally, in the majority of the aforementioned studies comparing LDLT and DDLT for HCC patients, tumor burdens such as the size, number, vascular invasion, and poor differentiation have proved to be independent risk factors for HCC recurrence after liver transplantation, all of which may lead to a rational explanation for the impaired recurrence - free survival of LDLT compared to DDLT.

OUR EXPERIENCE

At our institution, the University of Tokyo Hospital, a total of 423 adult recipients underwent LDLT by the end of 2012. Among them, 125 (30%) patients had HCC. The principle criterion for LDLT for HCC at our center is "up to 5 nodules with a maximum tumor diameter within 5 cm", which we call the "5-5 rule"[39]. Of the 125 patients, 118 (94%) were within the 5-5 rule criteria and 109 (87%) were within the Milan criteria. Overall survival of the 125 recipients at 1, 3, and 5 years was 88%, 82%, and 76%, respectively, with a median follow-up period of 8 years. A total of 11 (9%) patients developed HCC recurrence with a cumulative recurrence rate at 1, 3, and 5 years of 6%, 9%, and 11%, respectively.

We compared the graft regeneration rate between patients with HCC recurrence (n = 11) and those without recurrence (n = 114) to confirm the association of liver regeneration with HCC recurrence. The regeneration rate was calculated as follows: (graft volume at 3 mo after LDLT- initial graft volume)/initial graft volume × 100 (%). As shown in Table 2, there was no difference in the regeneration rate between those with HCC recurrence and those without recurrence. At the same time, the graft type (right *vs* left) and the initial graft volume ratio to the recipient's standard liver volume were also compared between groups, revealing no difference. A similar result was reported by the Asan group of South Korea[40], in which the graft-recipient weight ratio had no impact on HCC recurrence after LDLT among 181 LDLT recipients with HCC. Our result as well as the report of the Asan group clearly demonstrated that graft regeneration of the partial liver graft has no impact on HCC recurrence, at least in a clinical setting. The independent predictors for HCC recurrence in our series were tumors not within the 5-5 rule (Tokyo criteria), AFP level over 400 ng/mL, and desgamma-carboxy prothrombin levels over 200 mAU/mL.

CONCLUSION

In conclusion, there is no strong evidence to support higher HCC recurrence after LDLT than DDLT, and it may be reasonable to use different indication criteria for LDLT and DDLT, while there could be a potential bias in choosing the articles in the present study. LDLT should always be considered as a treatment option for HCC patients with advanced cirrhosis in areas where deceased donors are scarce or for patients whose tumor status interrupts access to DDLT.

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ORIGINAL ARTICLE

Use of simeprevir following pre-emptive pegylated interferon/ribavirin treatment for recurrent hepatitis C in living donor liver transplant recipients: a 12-week pilot study

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Abstract
Background The management of recurrent hepatitis C following liver transplantation remains a challenge.
Methods We prospectively investigated the efficacy and safety of simeprevir in combination with pegylated interferon and ribavirin in five patients undergoing living donor liver transplantation (LDLT) with recurrent hepatitis due to hepatitis C virus (HCV) genotype 1b.
Results As the immunosuppressive regimen, four received cyclosporine A (CsA) and one received tacrolimus (FK); no dose adjustment was made prior to the introduction of simeprevir, but the dose was accordingly modified afterwards. All five patients completed the intended 12-week treatment course without significant adverse events greater than grade 2, and no episodes of rejection were detected during the study period. The trough levels of CsA and FK were stably maintained. At week 12, HCV-RNA was not detectable in three of the five patients, whereas the HCV titer of the other two patients, including one with Q80L and V170I mutations at the HCV NS3 position, was at the lower level of quantification (1.2 $\log_{10}$ IU/ml).
Conclusions Based on this pilot study, simeprevir-based triple therapy is safe and somewhat effective within the first 12 weeks in LDLT recipients with HCV recurrence. Further studies are warranted to obtain robust conclusions.

Keywords Direct-acting antiviral drugs · Hepatitis C · Living donor liver transplantation · Simeprevir

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Introduction

Compared with liver transplant patients not infected with hepatitis C virus (HCV), those with HCV have a poorer post-transplant prognosis [1–3], especially when the virologic response is inadequate [4, 5]. The lower antiviral response in liver transplant recipients, however, limits the efficacy of conventional interferon-based antiviral treatment (pegylated interferon [Peg-IFN] and ribavirin [RBV]) for recurrent hepatitis C following liver transplantation [6].

In the past several years, the development of direct-acting antiviral drugs (DAA), telaprevir (TVR) and boceprevir (BOC), for the treatment of HCV genotype 1 has provided a promising treatment option [7, 8]. Although the feasible efficacy of triple therapy, including such "1st generation protease inhibitors", has been demonstrated by several groups, the likelihood and severity of adverse events seem to be inevitable and have limited its use as the first choice for recurrent hepatitis C post-liver transplantation [9]. In addition, it is difficult to maintain the levels of calcineurin inhibitors such as cyclosporine A (CsA) or tacrolimus (FK) in combination with the 1st generation DAA, which are primarily metabolized by the cytochrome P450 3A4 pathway [10].

In December 2013, simeprevir (SMV), which is a one-pill, once-daily, oral HCV NS3/4A protease inhibitor, a so-called "2nd generation protease inhibitor", was approved for clinical use in Japan. SMV is associated with few adverse events, but the antiviral effects in patients with hepatitis C are as good or better than those of DAA [8]. In liver transplant recipients, SMV is likely superior to prior DAAs in terms of drug interactions, based on its small impact on the blood levels of calcineurin inhibitors when used simultaneously [10].

We conducted this prospective pilot study to evaluate the feasibility of SMV-based triple therapy in liver transplant recipients with hepatitis C, mainly with respect to the antiviral response, adverse events, and drug interactions with immunosuppressants by week 12 (namely by the cessation of SMV).

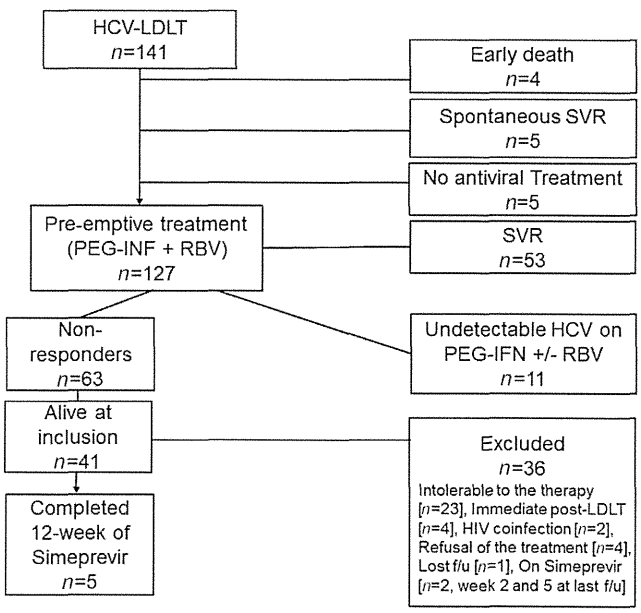


Fig. 1 Flow diagram of the patients enrolled in the simeprevir-based triple therapy

Materials and methods

Antiviral treatment regimen and patient selection

Between January 1996 and December 2013, 141 adult-to-adult living donor liver transplantations (LDLTs) were performed for HCV-positive recipients at the University of Tokyo Hospital. As previously reported [11], antiviral treatment was generally initiated with low-dose Peg-IFN alpha-2b and RBV 200–400 mg/day promptly after improvement of the general condition following liver transplantation in our institution. Recovery of hematologic and renal function was considered crucial, with a leukocyte number >4000/ml, platelet count >50000/ml, hemoglobin >8 g/l, and serum creatinine levels <2 mg/dl. During conventional dual treatment, flexible dose adjustments were made as necessary to avoid serious adverse events. A fixed overall treatment period length was not defined. Splenectomy was performed at the time of LDLT to prevent the progression of thrombocytopenia under IFN-based antiviral therapy [12].

Pre-emptive Peg-IFN /RBV treatment was administered to 127 of our 141 HCV-positive LDLT recipients, excluding cases of early death (within 3 months) after LDLT ($n = 4$), cases with spontaneous sustained virologic response (SVR) ($n = 5$), and cases without antiviral treatment due to clinical decision ($n = 5$). SVR was achieved in 53 patients, 11 had undetectable HCV-RNA on Peg-IFN and RBV therapy (dual treatment) upon inclusion; the remaining 63 were classified as non-responders. We selected patients for the current study among the 41 non-responders who were alive with sustainably positive HCV-RNA at the time of inclusion in this study. Patients who had either not tolerated or were not expected to tolerate conventional dual treatment were excluded. The current study protocol was not intended for those who were immediately post-transplant or were coinfected with human immunodeficiency virus (HIV) because of the lack of a detailed profile of SMV-based triple therapy in the transplant setting, considering the risk of unknown adverse events that could be fatal in this population, but only for those who survived the perioperative period and tolerated dual therapy for recurrent hepatitis C. Patient selection is shown in the flowchart in Figure 1.

SMV (100 mg daily) was intended to be continued for 12 weeks in combination with Peg-IFN and RBV (triple-antiviral treatment), followed by 36 weeks of dual treatment. The patients were generally admitted for 1 week, both to undergo liver biopsy pre-induction of SMV and to carefully monitor the daily change in the trough levels of calcineurin inhibitors (CNIs) following the induction of SMV.

Here we prospectively studied the 12-week clinical courses of all five patients who met the inclusion criteria and in whom triple-antiviral therapy with SMV was initiated by the end of March 2014, and followed up by the end of June 2014.

Laboratory test and histopathology assessment

Conventional blood work for the management of the patients with post-transplant hepatitis was checked as necessary. The estimated glomerular filtration rate (eGFR; ml/min per 1.73 m^2) was calculated using the following formula: 194 × serum creatinine (−1.094) × age (−0.287) × 0.739 (if female), Japanese equation (equation 4) [13]. HCV RNA was measured quantitatively by reverse-transcriptase polymerase chain reaction (Amplicor HCV; Roche Molecular Systems, Pleasanton, CA, USA). Before liver transplantation, the HCV genotype was determined: the HCV genotype in all the five patients was 1b. In addition, the nucleotide sequences of the core and the number of amino acid substitutions in the interferon sensitivity-determining region (ISDR) in the NS5A gene were determined using a direct sequencing method [14]. The interleukin 28B (IL28B) genotype rs8099917 was also examined using the Invader assay (Third Wave Technologies, Madison, WI, USA) [15]. Prior to the induction of SMV, HCV NS3 and NS5A sequencing was determined, and liver biopsy was performed and evaluated by a pathologist based on the Metavir score [16].

Immunosuppression

Our post-transplant strategy for immunosuppression is documented elsewhere [11, 17]: briefly, it comprises steroid induction with CsA or FK, and the doses of each drug are gradually tapered for 6 months after LDLT. Methylprednisolone is tapered from 3 mg/kg on the first postoperative day to 0.05 mg/kg at the sixth postoperative month, and a maintenance dose of 2–4 mg of methylprednisolone is continued in all recipients. Mycophenolate mofetil (MMF) is added mainly for recipients requiring CNI dose reduction.

Ethics statement

The study protocol was approved as project number 2032, and human subject research regarding the IL28 polymorphism was particularly approved as project number G3514 by the Graduate School of Medicine and Faculty of Medicine at the University of Tokyo Research Ethics Committee; and the Human Genome, Gene Analysis Research Ethics Committee.

Statistical analysis

We used SPSS 17.0 statistical software (SPSS, Chicago, IL, USA) to analyze the relevant data. Differences between groups were analyzed by the Mann–Whitney *U*-test or ANOVA for continuous variables as appropriate, and the χ^2 test for categorical variables. *P*-values <0.05 were considered significant.

Results

The clinical characteristics of those five LDLT recipients are shown in Table 1. The median Model for End-Stage Liver Disease score was 15 (range 9–23). None of the five was coinfected with HIV, and four (80%) had hepatocellular carcinoma within the Milan criteria [18]. The details of each patient, including the HCV profile and the single nucleotide polymorphisms of IL28B rs8099917, are shown in Table 1. The Q80L/V170I and S122T/V170 mutations in NS3 were detected in patient #2 and 3, respectively. Q54H, F37L, Q54H, F37L/Q54H/Q62E, F37L mutations in NS5A were detected in patient #1 to 5, respectively.

Efficacy

All five patients completed the 12-week course of triple therapy with SMV. All of them were treated with dual therapy with Peg-IFN and RBV afterward.

Three of the five patients achieved an undetectable viral load of HCV at week 4, 8, and 12 weeks, and the viral titer of the remaining two patients was at the lower level of quantification (LLOQ, <1.2 $\log_{10}$ IU/ml) at week 4; one patient achieved an undetectable viral load at week 8, but the viral load became detectable again at week 12. The HCV titer of the remaining patient remained around LLOQ at weeks 8 and 12 (Table 1). At the last follow up (median 22 [range 16–27] weeks since the initiation of triple therapy), HCV viral load of those with undetectable HCV-RNA at week 12 were sustained to be below detectable level, although those with positive HCV-RNA at week 12 were both positive then (1.4 and 7.5 $\log_{10}$ IU/ml). HCV-RNA levels in the five patients are shown in Figure 2.

Safety profile and immunosuppression levels with SMV

No significant adverse events were observed other than grade 2 diarrhea in patient #1 on day 26, which was resolved immediately (within 1 week) after the reduction of mycophenolate mofetil (MMF) from 3000 mg/day to 1500 mg/day. None of the five patients required a dose reduction of Peg-IFN or RBV, use of granulocyte-colony stimulating factor for neutropenia, or blood transfusion for anemia. Renal function was well preserved during the study period, with no significant change in eGFR before or after the introduction of SMV (median 68 [range, 39.1–97.2] to 64.9 [range, 44.5–102] ml/min, $P = 0.84$). Bilirubin levels were not increased in any of the five patients. Immunosuppression was not modified before the initiation of SMV. The CsA trough levels before (median 78 [range 48–113] ng/ml), 1 week after (median 68.5 [67–104] ng/ml) and 12 weeks after (median 72.5 [65–92] ng/ml) initiating the triple therapy did not differ significantly ($P = 0.72$), and the FK

Table 1 Patient characteristics

Patient #	1	2	3	4	5
Age (years)	51	64	66	49	59
Sex	M	F	M	M	F
Height (cm) / weight (kg)	170/65	147/54	166/56	168/63	156/53
Donor age (years)	50	30	24	44	60
Donor relationship	Spouse	Daughter	Son	Spouse	Spouse
Calcineurin inhibitor (mg/day)	CsA (40)	CsA (75)	CsA (60)	FK (2)	CsA (60)
MMF (mg/day)	3000	None	1000	1500	None
Histopathological activity and fibrosis at triple therapy[a]	A2 / F1	A0-1 / F0-1	A1 / F1	A0 / F0	A1 / F1
Baseline clinical chemistry at triple therapy					
Total bilirubin (mg/dl)	1.9	0.8	0.9	0.9	0.7
Alanine aminotransferase (IU/ml)	68	31	47	25	29
Creatinine (mg/dl) and Estimated GFR (ml/min)	0.65 / 100.5	0.64 / 70.5	1.43 / 39.4	1.33 / 46.2	0.61 / 76
International normalized ratio	1.29 (on warfarin)	0.90	0.85	0.95	0.84
Hemoglobin (g/dl)	9.0	8.5	12.3	13.5	9.6
Leukocytes (/ul)	5900	5000	4900	5900	4600
Platelets (/ul)	476000	145000	186000	192000	262000
NS3 mutation	Non	Q80L/V170I	S122T/V170I	Non	Non
NS5A mutation	Q54H	F37L	Q54H	F37L/Q54H/Q62E	F37L
Pre-transplant antiviral therapy	Relapse	Non responder	Not applicable	Not applicable	Not applicable
Baseline HCV-RNA pre-LT ($\log_{10}$ IU/ml)	3.1	6.4	7.1	6.7	5.7
TPV therapy post -LT	Relapse	Not applicable	Relapse	Not applicable	Not applicable
Pre-triple treatment interferon (mo) since LT	23	16	118	26	16
Dose of Peg-IFN α2b (μg/week)	80	70	100	100	100
RBV dose (mg/day)	200	200	200	200	200
%CNI after the triple therapy	50%	67%	100%	75%	100%
CNI trough at triple therapy (ng/ml)	113	73	48	9.8	83
CNI trough 1 week after initiation (ng/ml)	104	69	67	9.5	68
CNI trough 12 week after initiation (ng/ml)	92	79	66	9.0	65
ISDR mutation (number)	Mutant (9)	Wild (0)	Wild (0)	Intermediate (1)	Undeterminable
Core 70	Undeterminable	Wild	Mutant	Wild	Wild
Core 91	Undeterminable	Wild	Mutant	Wild	Wild
IL28B Recipient /Donor[b]	TT/TT	TG/TT	TG/TT	GG/TG	TT/TT

CNI calcineurin inhibitor, *CsA* cyclosporine A, *FK* tacrolimus, *GFR* glomerular filtration rate, *HCV* hepatitis C virus, *IFN* interferon, *MMF* mycophenolate mofetil, *RBV* ribavirin, *LT* liver transplantation

[a] As per Metavir

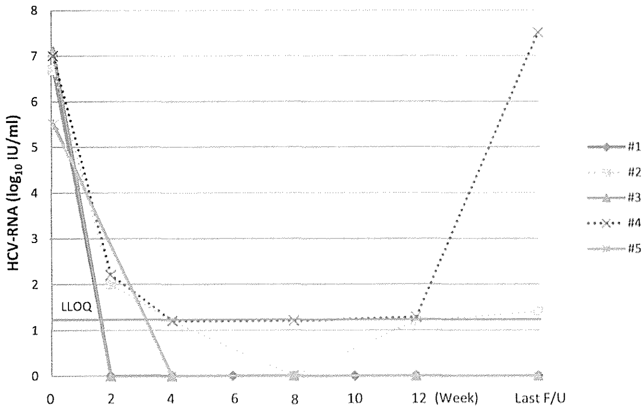
[b] Genotype rs8099917

trough level only moved from 9.8 to 9.0 ng/ml following the initiation of SMV.

After the completion of SMV, the CNIs were not restored to the original dose automatically, but modified according to the trough levels. Those without dose adjustment during the triple therapy (patient #3 and 5), the trough level at the last follow up were stable (67 and 61 ng/ml, respectively) with the same dose of CsA. Patient #2 showed lower trough level at week 20, and the dose of the CsA was re-increased to the original dose (75 to 100 ng/ml). The CNI dose of the remaining two patients (patient #1 and 4) were not changed since the completion of SMV to the last follow up with stable trough levels. The dose/use of MMF was not changed during the triple therapy throughout the follow up period, other than patient #1 who experienced diarrhea as noted above. There were no episodes of acute cellular or chronic (ductopenic) rejection observed during the study period.

Discussion

Here we present the results of a pilot study to reveal the characteristics of SMV-based triple anti-HCV treatment for



	w0	w4	w8	w12	Last F/U
#1	6.7	Undetectable	Undetectable	Undetectable	(w27) Undetectable
#2	6.7	<1.2	Undetectable	<1.2	(w23) 1.4
#3	7.1	Undetectable	Undetectable	Undetectable	(w22) Undetectable
#4	6.7	<1.2	<1.2	1.2	(w21) 7.5
#5	5.5	Undetectable	Undetectable	Undetectable	(w16) Undetectable

Fig. 2 Hepatitis C virus (HCV) RNA levels in five patients with simeprevir-based triple antiviral treatment. Each solid line represents an individual patient with an on-treatment virological response. Each dashed line represents an individual patient who did not achieve undetectable HCV RNA at week 12. The lower level of quantification (LLOQ) was 1.2 log10 IU/ml

LDLT recipients with recurrent hepatitis C. SMV became available after the introduction of TVR, which we have used in a selected patient group before the SMVs were introduced, and BOC into the liver transplant setting, thus a primary aim of the present study was to provide a preliminary report of the clinical experience with SMV in the liver transplant setting. Compared with TVR and BOC, the result of the current study suggested that the treatment with SMV was acceptably effective, with a rapid virologic response in three out of all five patients. In addition, importantly, no fatal adverse events, such as rejection, renal impairment, or severe cytopenia were observed.

We treated patients with SMV-based triple therapy as part of the pre-emptive therapy for recurrent hepatitis C. The rationale for this pre-emptive therapy is to strike at a time when histologic damage is minimal regardless of the clinical symptoms of recurrent HCV following transplantation [11, 19, 20]; thus we initiated SMV for those with even minimal or no graft injury due to recurrent hepatitis C, as long as the HCV remains persistent with dual treatment.

We investigated HCV polymorphisms at the NS3 position in all patients before the introduction of SMV. At baseline, none of the patients had mutations reported to reduce the antiviral effects of SMV *in vitro* [21]. Patient #2 had Q80L and V170I mutations at baseline; she achieved an undetectable HCV titer at week 8, whereas the other three patients achieved an undetectable HCV titer within the first 4 weeks, including two patients who relapsed with TPV-based triple therapy prior to the current study. The HCV-RNA of patient #2 became positive again at week 12, although it was around the LLOQ and not regarded as a breakthrough.

We also checked baseline polymorphisms at the NS5A position at the same time in anticipation of the coming treatment option with Daclatasvir (first-in-class, NS5A replication complex inhibitor) combined with Asunaprevir (NS3 protease inhibitor), which has been well tested in phase 3 clinical trial in Japan [22]. Patient #1, 3 and 4 had the Q54H mutation in NS5A, which might be associated with low-level resistance to an NS5A replication complex inhibitor [23]. Two out of those three patients achieved early virologic response. It seems feasible to introduce SMV-based triple therapy for such patients especially with some doubts about the potential efficacy of dual therapy with Daclatasvir and Asunaprevir in the liver transplant setting.

Importantly, there were no treatment cessations due to side-effects. One patient experienced grade 2 diarrhea, but this was resolved soon after the reduction of MMF: thus, it is difficult to determine whether SMV was the risk factor for diarrhea. Otherwise, no significant adverse events were observed, including elevation of serum total bilirubin. Necessary modifications in immunosuppression, especially CNIs, were also minimal. Technically it was not difficult for us to safely modify the dose of CNIs without a dose adjustment prior to the introduction of SMV, and comparatively mild modifications (50% to none) were required during the triple therapy. None of the five patients experienced renal dysfunction, infection, or rejection due to the uncontrolled trough level of CNIs, as noted above.

The introduction of TVR and BOC was anticipated to greatly improve virologic effects, even in liver transplant recipients with recurrent hepatitis C. The efficacy of TVR- or BOC-based triple therapy, however, was somewhat unsatisfactory; approximately 50% of the patients receiving such treatment achieved SVR [9, 24–27]. TVR- or BOC-based triple therapy was also associated with challenges in controlling the CNI trough levels and unignorable adverse events, such as cytopenic events, renal impairment, or skin rash [9]. In contrast, the previously reported profile of SMV is promising for liver transplant recipients with recurrent hepatitis C for the following reasons: first, the virologic effect is much greater than that of only Peg-IFN and RBV, with few side-effects by SMV itself [8, 28, 29], and second, SMV has few drug interactions with CNIs [10]. As demonstrated in the present study, the reported advantages of SMV in addition to TVR or BOC seem to be applicable to the management of post-transplant recurrent hepatitis C, with its safety and feasible virologic effect compared to TPV and BOC.

The present study has several limitations. The number of patients included was limited to only five, and all five patients were selected from among those receiving pre-emptive antiviral therapy following liver transplantation with a poor virologic response. In addition, the five patients showed minimal or no graft damage when SMV was started. Hence, this study does not allow us to draw a robust conclusion regarding the use of SMV for liver transplant recipients, especially in evaluating the potential efficacy of SMV as a first-line treatment for recurrent hepatitis C. In addition, patients were followed only during the SMV-based triple therapy, and the actual virologic response after completing the treatment (i.e., 36 more weeks of dual therapy with Peg-IFN and RBV) should be evaluated. Further studies are warranted to address those concerns.

In conclusion, the present pilot study revealed the feasibility and safety of SMV in combination with Peg-IFN and RBV in LDLT recipients with recurrent hepatitis C. This combination therapy produced fewer side-effects and drug interactions with CNIs than prior DAAs. Recipients who were tolerant to dual therapy (Peg-IFN with RBV) but could not achieve a satisfactory viral response should be considered candidates for SMV. The actual profile of the current SMV-based antiviral treatment for recurrent hepatitis C post-liver transplantation, however, should be evaluated after the completion of a full course of therapy followed by 36 weeks of dual therapy with Peg-IFN plus RBV. In addition, future studies including a larger number of liver transplant recipients in diverse situations, such as those undergoing first-line treatment for established recurrence of HCV post-liver transplantation, are crucial.

Conflict of interest None declared.

Author contribution Study design: Tomohiro Tanaka, Yasuhiko Sugawara and Norihiro Kokudo. Acquisition of data: Nobuhisa Akamatsu, Junichi Kaneko, Sumihito Tamura, Taku Aoki, Yoshihiro Sakamoto, Kiyoshi Hasegawa. Analysis and interpretation: Tomohiro Tanaka, Masayuki Kurosaki, Namiki Izumi and Yasuhiko Sugawara. Manuscript drafted by: Tomohiro Tanaka, Nobuhisa Akamatsu, Masayuki Kurosaki and Yasuhiko Sugawara. Study supervision: Norihiro Kokudo.

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Study of Education Program of In-Hospital Procurement Transplant Coordinators in Japan

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ABSTRACT

Background. As the number of donated organs is still extremely small in Japan compared with other developed countries, in-hospital procurement transplant coordinators (In-Hp PTC) may play an important role in increasing organ donation and making the procurement procedure smoother. In this study, our education program of In-Hp PTC is described.

Materials and Methods. In May 2012, our department started the In-Hp PTC Education Program. In the first semester, a 2-hour lecture is provided every 2 weeks for 5 months to 15 In-Hp PTCs working near Osaka. In the second semester, 20 lectures were provided for 3 consecutive days to 31 In Hp PTCs, more than 80% of whom work far from Osaka. Lecture topics were the history and current status of organ donation in Japan, social regulation of organ donation, care of transplant recipients, overall procedures of organ donation, the role of In-Hp PTC, donor family care, donor indications, and donor assessment and management. Lectures also included simulations of the organ donation process.

Results. Participants were surveyed for their opinions after the program, Most participants were satisfied with the program, topics and duration. As most of them are not full-time In-Hp PTCs, they preferred to attend the 3-day program. Many participants are currently working as main In-Hp PTCs and establishing their own organ donation system in their hospital.

Conclusions. In-Hp PTCs have an important role to play in establishing an organ procurement system and increasing organ donation in Japan. This program may help establish a systematic education program for this occupation in Japan.

THE JAPANESE Organ Transplantation Act for brain-death (BD) organ donation (the former Act) was issued in October 1997. It required a living written consent for BD and other organ donation and did not allow BD donation from children younger than 15 years. For these reasons, only 81 BD organ donations were performed in Japan for 13 years after the issuance of the former Act. In 2007, the cardiac donation rate per million population in Japan was only 0.08, compared with 7.3 in the U.S., 5.3 in Spain, and 0.97 in South Korea.

The Act was finally revised on July 17, 2010 [1–3]. According to the new law, organs may be donated after BD with the consent of the family as long as the BD patient did not refuse organ donation. Although BD organ donation increased from 13 to 47 cases a year after the law was revised, the number was still much smaller than in other developed countries. The revised law allows organ donation from BD children younger than 15. However, only 158 of 504 (31.3%) procurement hospitals in which BD organ donation is allowed by the Government have established systems to procure organs from children. In these circumstances, In-Hp procurement transplant coordinators (PTCs) may play important roles in increasing organ donation and making procurement procedure smoother.

The Department of Coordinators and the coordinator committee in the Japan Organ Transplant Network (JOT), the only organ procurement organization (OPO) in Japan, play a main role in educating JOT PTCs. JOT has made guideline manuals of standard roles and procedures of PTC during organ procurement in BD donors and

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donation after cardiac death [4]. Although JOT created a textbook and held several educational programs, In-Hp PTCs have been educated mainly by prefectural PTC or by their own hospital. But, these educational programs for In-Hp PTC usually consist of annual or biannual lectures of only 1 or 2 topics and lecture time is less than 3 hours. There has been no systematic education program for In-Hp PTC in Japan. Therefore, the education system for In-Hp PTCs needs to be modified.

To establish an effective education system for In-Hp PTCs, their current status and needs should be clarified. So we undertook a nationwide survey between December 15, 2011, and January 31, 2012 [5]. We invited 1889 In-Hp PTCs to complete a letter survey using a 28-item self-designed questionnaire. In summary, it was very difficult for them to do their daily activities, to manage a rare and sudden donation case and to find time to learn about organ donation because they had other jobs. Although 77% have attended seminars about organ donation organized by JOT, the prefecture PTC, academic associations for organ or tissue transplantation and the Japan Transplant Coordinator Organization (JATCO), 93% still wanted more professional education. Nurses and medical social workers were more likely to want more professional education than physicians and medical examiners.

The topics they wanted to learn about were donor family care, overall procedures of organ/tissue donation, the role of the In-Hp PTC, how to project simulation of organ donation, the legislation and social system of organ donation, medical indications for organ donation, the current status of organ donation and transplantation in Japan, donor assessment and management, and case studies. There were significant variations in topics of interest among different occupations.

From the result of this nationwide survey, our department started to hold special educational seminars for In-Hp PTCs and established a educational program for In-Hp PTCs in Japan in May 2012. Participants who attended more than 60% of the lectures and scored above 60% in the written examination held on the last day received a Certificate of completion of course from the Rector of Osaka University.

In the present study, our Education Program of In-Hp PTC is described and the future of education program of In-Hp PTC in Japan is discussed.

MATERIALS AND METHODS

In May 2012, our department began the In-Hp PTC Education Program. In the first semester, 2-hour lectures were provided every 2 weeks for 5 months to 16 In-Hp PTCs, all of whom worked near Osaka. In the second semester, 20 lectures were provided for 3 consecutive days between Friday and Sunday to 31 In-Hp PTCs, more than 80% of whom worked far from Osaka. In both semesters, nearly 80% of participants were nurses and only one third and one fourth of the participants had experienced BD organ donation in their hospital, respectively (see Table 1).

Lecture topics (see Tables 2 and 3) were history of organ transplantation in Japan and in the world, the current status of organ donation and transplantation in Japan, the social regulation of organ donation, care of transplant recipients, overall procedures of organ donation (brain dead and donation after cardiac death), the role of In-Hp PTCs, donor family care, donor indications, and donor assessment and management. The program included a role-play study of how to organize simulation seminars in each hospital and how to present an option for organ donation, as well as group discussion of bereaved family care using case studies. Therefore, all participants in the program could learn about all of the topics that the responders of the nationwide survey wanted to learn about.

A 1-day follow-up course was also organized at the end of second semester. Thirteen of 46 participants at the first and second seminars attended. The course included a lecture titled "Recent Status of Organ Donation in Japan" and a presentation by a neurosurgeon and two nurses about their experiences with organ donation in their hospital after having taking the first or second semester course.

Table 1. Characteristics of the Participants

	First Semester (n = 15)	Second Semester (n = 31)
Occupation		
Nurse	12	26
Physician	1	2
Medical social worker	1	1
others	1	2
Working area		
Osaka	13	4
Near Osaka (<50 km)	2	2
Far (50–200 km)	0	12
Very far (200–700 km)	0	5
Too far (>700 km)	0	8
Experience of brain death organ donation		
Yes	5	8
None	10	23

Table 2. Education Program for 10-Day Course for 1st Semester (Over 5 Months)

	Contents
Day 1	① History of organ donation and transplantation in Japan
	② Organ Transplant Act and Network network System in Japan
Day 2	② Outcome of organ transplantation in Japan
	④ Post-transplant nursing care
Day 3	③ Brain death (pathology and legal determination)
	⑥ Roles of procurement transplant coordinator (PTC)
Day 4	⑦ Flow of organ donation, site visit to JOT office
Day 5	⑧ Family approach by JOT-PTC
	⑨ Family care (national survey of DCD donor family) (role play)
Day 6	⑩ Donor assessment and management, and management in OR
Day 7	⑪ Roles of In-Hp PTC
	⑫ How to organize simulation seminars in each hospital
Day 8	⑬ Pediatric organ donation, how to deny child abuse
	⑭ How to present an option for organ donation
Day 9	⑮ Grieving process of families (role play)
Day 10	⑯ Summary and written examination