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Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma

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To identify the genetic susceptibility factor(s) for hepatitis C virus–induced hepatocellular carcinoma (HCV-induced HCC), we conducted a genome-wide association study using 432,703 autosomal SNPs in 721 individuals with HCV-induced HCC (cases) and 2,890 HCV-negative controls of Japanese origin. Eight SNPs that showed possible association ($P < 1 \times 10^{-5}$) in the genome-wide association study were further genotyped in 673 cases and 2,596 controls. We found a previously unidentified locus in the 5′ flanking region of *MICA* on 6p21.33 (rs2596542, $P_{combined} = 4.21 \times 10^{-13}$, odds ratio = 1.39) to be strongly associated with HCV-induced HCC. Subsequent analyses using individuals with chronic hepatitis C (CHC) indicated that this SNP is not associated with CHC susceptibility ($P = 0.61$) but is significantly associated with progression from CHC to HCC ($P = 3.13 \times 10^{-8}$). We also found that the risk allele of rs2596542 was associated with lower soluble MICA protein levels in individuals with HCV-induced HCC ($P = 1.38 \times 10^{-13}$).

It is estimated that more than 170 million people are infected with HCV worldwide[1]. Persistent HCV infection causes CHC and, subsequently, fatal liver diseases such as liver cirrhosis and HCC. Therefore, the treatment of HCV carriers is an issue of global importance. HCC is the third most common cause of cancer-related deaths[2], and HCV infection accounts for 30–70% of the individuals with HCC[3,4]. HCV-induced HCC is a multistep and progressive liver disease in which disease progression may be influenced by both environmental and genetic risk factors. The impact of host genetic variation on progression to CHC after HCV exposure is well documented by recent genome-wide association studies (GWAS)[5–7]. However, no comprehensive analyses have been performed to explore the genetic basis of HCV-induced HCC. Therefore, we conducted a GWAS for HCV-induced HCC.

We genotyped the DNA of 721 individuals with HCV-induced HCC and 2,890 HCV-negative controls (**Supplementary Table 1**) from BioBank Japan[8]. After the initial standard SNP quality filters, we obtained genotyping results for 432,703 SNPs for association analysis. Because progression from CHC to liver cancer is strongly affected by age and gender[3], we performed a logistic regression analysis by including age and gender as covariates at all tested loci in our analyses. The genetic inflation factor (λ) was 1.03, indicating that there is no or little population stratification (**Supplementary Fig. 1**). Although no SNPs cleared the GWAS significance threshold ($P < 5 \times 10^{-8}$) at this stage, we identified eight independent loci showing possible association ($P < 1 \times 10^{-5}$; **Supplementary Fig. 2**).

In the replication stage, 673 cases from an independent HCC cohort from the University of Tokyo and 2,596 HCV-negative controls from BioBank Japan were genotyped at these eight SNPs. We observed a significant replication of association at rs2596542 on chromosome 6p21.33 ($P = 8.62 \times 10^{-9}$, odds ratio (OR) = 1.44, 95% confidence interval (CI) 1.27–1.63; **Table 1**), whereas the remaining seven SNPs failed to replicate the association (**Supplementary Table 2**). Furthermore, the combination analysis of the GWAS and replication study data at rs2596542 revealed a highly significant association in which the frequency of the risk allele A is higher in cases ($P = 4.21 \times 10^{-13}$, OR = 1.39; **Fig. 1** and **Table 1**) after the age and gender adjustment, without any heterogeneity ($P = 0.24$) between the two stages. To further investigate the impact of rs2596542 on the complex nature of the HCV-induced HCC phenotype, we genotyped 1,730 individuals with CHC who had not developed liver cirrhosis or HCC during their recruitment. As a result, rs2596542 was found to have no association with chronic hepatitis C susceptibility ($P = 0.61$) but was significantly associated with progression from CHC to HCC ($P = 3.13 \times 10^{-8}$, OR = 1.36; **Table 2**).

Because heavy alcohol consumption (>50 g per day) as well as poor response to interferon (IFN) treatment were shown to be the major risk factors for HCC among individuals with CHC[9], we evaluated the effect of alcohol consumption as a confounding factor and found that rs2596542 remained highly significant even after adjustment for this factor (non-HCV versus HCC, OR = 1.39, $P = 1.22 \times 10^{-11}$; CHC versus HCC, OR = 1.25, $P = 2.31 \times 10^{-4}$; **Supplementary Table 3**). The major genotypes of HCV can be determined by a serotyping

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Table 1 Association results of rs2596542 in the GWAS, replication stage and combined analysis

SNP	Chr. (locus)	Stage	Case RAF	Control RAF	*P*	OR (95% CI)
rs2596542 (A/G)	6 (*MICA*)	GWAS[a]	0.388	0.331	4.50×10^{-6}	1.34 (1.16–1.53)
		Replication[a]	0.413	0.331	8.62×10^{-9}	1.44 (1.27–1.63)
		Combined[a]	0.400	0.331	4.21×10^{-13}	1.39 (1.27–1.52)
				MH test	7.76×10^{-12}	1.35 (1.24–1.47)

We analyzed 1,394 cases with HCC (721 in the GWAS and 673 in the replication) and 5,486 controls (2,890 in GWAS and 2,596 in replication). Chr., chromosome; RAF, risk allele frequency (allele A); OR, odds ratio for the minor allele calculated by considering the major allele as a reference; MH, Mantel-Haenszel.
[a]*P* values and ORs are adjusted for age and gender by logistic regression analysis under an additive model.

assay that is based on the type-specific antibodies produced by the infected host[10]. A subgroup analysis for HCV serotypes or history of IFN therapy indicated that this variation is associated with HCC susceptibility independently of HCV genotypes or treatment response (**Supplementary Fig. 3**). Consistent with this result, rs1051796, which had $r^2 = 0.7$ and $D' = 0.95$ with rs2596542, was not associated with IFN response ($P = 0.89$) according to previously published data in the Japanese population[11].

rs2596542 is located within the class I major histocompatibility complex (MHC) region. The human MHC region encompasses the complex and extended linkage disequilibrium (LD) structure[12,13]. Several HLA alleles and genes within MHC region have been implicated in HCV infection or clearance or in response to treatment[14–16]. Therefore, we searched the whole 7.5-Mb extended MHC region using GWAS data to test the possibility of other associated loci. We found a moderate association peak at rs9275572 ($P = 4.99 \times 10^{-5}$), which is located between *HLA-DQA* and *HLA-DQB* loci (**Supplementary Fig. 4**). Subsequent replication and combination analyses at rs9275572 indicated a significant association with HCV-induced HCC ($P = 9.38 \times 10^{-9}$, OR = 1.30; **Supplementary Table 4**). The multiple logistic regression analysis to control for alcohol consumption along with age and gender also indicated a significant association at rs9275572 ($P = 3.21 \times 10^{-8}$, OR = 1.29; **Supplementary Table 5**). However, rs2596542 was not in high LD with rs9275572 ($D' = 0.41$, $r^2 = 0.16$), and both SNPs remained associated with HCC even after conditional analysis on each other and had small reductions in their ORs upon conditioned analysis (OR = 1.23, $P = 4.43 \times 10^{-6}$ and OR = 1.17, $P = 0.00059$, respectively; **Supplementary Table 6**). A haplotype analysis between these two markers showed four possible haplotypes, with haplotype AA showing higher risk (with OR = 1.44) compared to the major haplotype GG (**Supplementary Table 7**). However, the OR for the risk haplotype was 1.32 with $P = 2.31 \times 10^{-10}$ after comparing against all observed haplotypes in the population (**Supplementary Table 7**), which is weaker than that of rs2596542 alone (OR = 1.39, $P = 4.21 \times 10^{-13}$). Hence, the impact of rs2596542 is much stronger than the haplotype of two SNPs, suggesting that rs2596542 is a principal genetic factor in this region. We also found that rs9275572 has a moderate association with CHC susceptibility as well as progression from CHC to HCC ($P = 0.03$ and $P = 2.58 \times 10^{-5}$, OR = 1.09 and OR = 1.29, respectively; **Supplementary Table 8**). Because *HLA-DQ* and *HLA-DR* alleles were shown to be associated with viral persistence and early liver disease among Japanese individuals[16], further study will be needed to confirm whether the association at rs9275572 is because of its LD with *HLA-DQ* or *DR* alleles.

In this regard, it is interesting to note that rs9275572 had a very strong expression quantitative trait locus effect on *HLA-DQB1* ($\log_{10}$ odds (LOD) ≥ 19.48) and *HLA-DRB4* alleles (LOD ≥ 26.88)[17]. Thus, it will be important to test the functional effect of the common haplotype (AA; **Supplementary Table 7**), which tags the risk alleles at these two SNPs.

Two SNPs, rs12979860 and rs8099917, at the *IL28B* locus were reported to be associated with spontaneous clearance of HCV virus[18] and response to pegylated IFN-α and ribavirin therapy[11], respectively. However, we found no association at rs12979860 and rs8099917 in our dataset (**Supplementary Table 9**). Because we used non-HCV control subjects rather than subjects who had cleared HCV infection spontaneously, and because only about 20% of the cases with HCC had been treated with IFN, our study may not be suitable to detect associations at the *IL28B* locus. In addition, the protective C allele at rs12979860 is nearly fixed throughout east Asia, with a frequency of more than 91% in the Japanese population as compared to 67% in European Americans[6], indicating a role for other factors in spontaneous clearance.

The top associated SNP, rs2596542, is located 4.7 kb upstream of *MICA*, the MHC class I polypeptide-related sequence A gene, and 41.7 kb downstream of the *HLA-B* gene (**Supplementary Fig. 5**). The regional association plot at the rs2596542 locus, made using genotype data from the GWAS (**Fig. 1**) and imputation analysis (**Supplementary Fig. 6**), revealed that all of the modestly associated SNPs are tightly

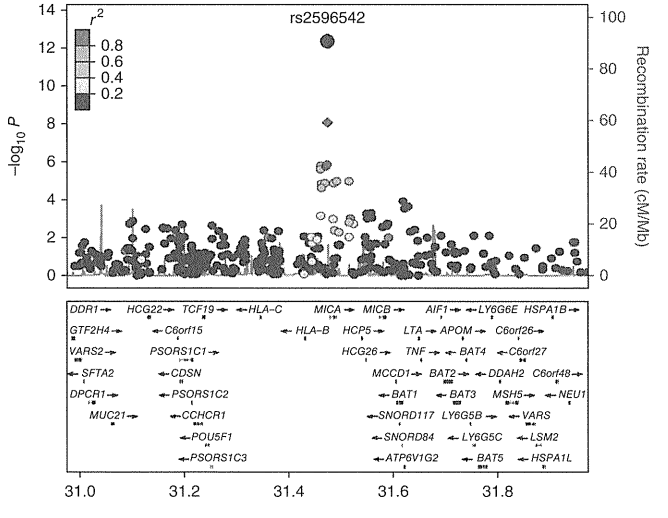


Figure 1 Regional association plot at rs2596542. Above, the *P* values of genotyped SNPs are plotted (as $-\log_{10}$ values) against their physical position on chromosome 6 (NCBI Build 36). The *P* value for rs2596542 at the GWAS stage, replication stage and combination analysis is represented by a purple diamond, circle and diamond, respectively. Estimated recombination rates from the HapMap JPT population show the local LD structure. Inset, the SNP's colors indicate LD with rs2596542 according to a scale from $r^2 = 0$ to $r^2 = 1$ based on pairwise r^2 values from HapMap JPT. Below, gene annotations from the UCSC genome browser.

Table 2 rs2596542 (A/G) is associated with progression from CHC to HCC

Subjects	RAF	(Comparison) *P*[a]	OR[a]	95% CI
Healthy	0.331			
CHC	0.333	(Healthy vs. CHC) 0.61	1.02	0.94–1.10
HCC	0.398	(CHC vs. HCC) 3.13×10^{-8}	1.36	1.22–1.51

We analyzed 5,486 controls, 1,730 CHC cases and 1,394 HCC cases. RAF, risk allele frequency (allele A); OR, odds ratio for the minor allele by considering the major allele as a reference.
[a]Calculated by logistic regression analysis, by PLINK upon age and gender adjustment under additive model.

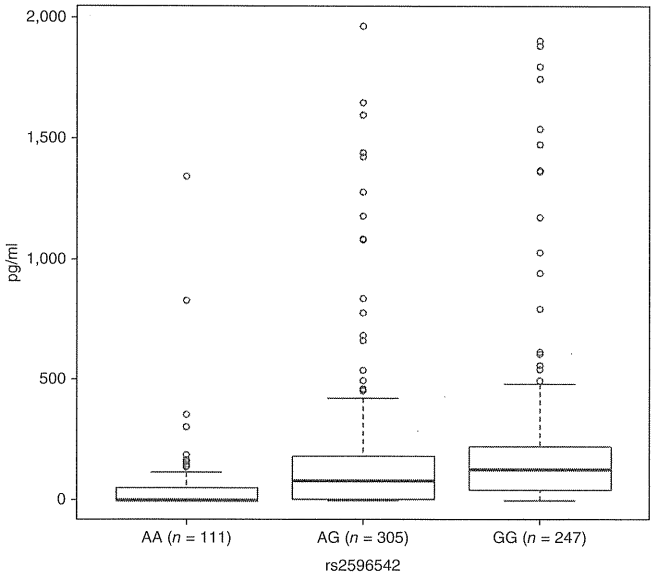
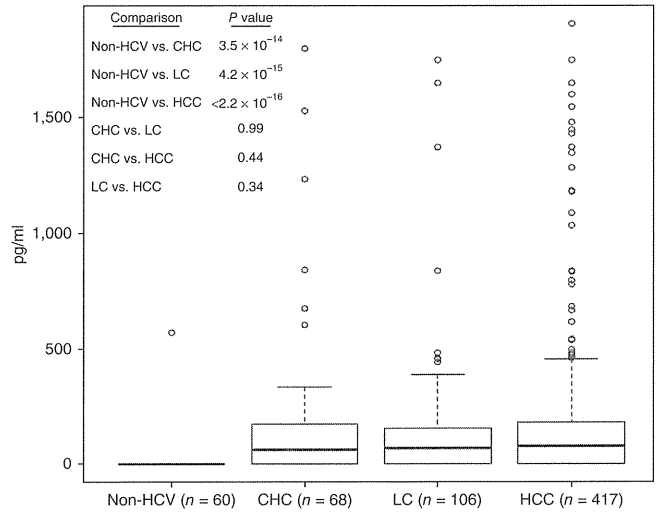


Figure 2 Correlation between soluble MICA levels and rs2596542 genotype. The *x* axis shows the genotypes at rs2596542, and the *y* axis shows the concentration of soluble MICA in pg/ml. The number of independent samples tested in each group is shown in parentheses. Each group is shown as a box plot, and the median values are shown as thick dark horizontal lines (median values of AA = 0, AG = 43.6 and GG = 77.74). The box covers the twenty-fifth to seventy-fifth percentiles, and the whiskers outside the box extend to the highest and lowest value within 1.5 times the interquartile range. Points outside the whiskers are outliers. We tested the difference in the median values among genotypes using the Kruskal-Wallis test ($P = 1.6 \times 10^{-13}$). We plotted the box plots using default settings in R (see URLs).

linked to rs2596542 ($r^2 > 0.4$) and are confined to the *MICA* gene locus. On the other hand, the imputation analysis of *HLA*-tagging SNPs did not show any evidence of linkage with rs2596542 (Online Methods and **Supplementary Table 10**), suggesting that *MICA* is a disease-associated candidate gene at this locus.

MICA is a membrane protein that acts as a ligand for NKG2D to activate anti-tumor effects through natural killer cells and $CD8^+$ T cells[19]. On the other hand, MICA is secreted into the serum by cleavage at the transmembrane domain with matrix metalloproteinases[20,21] and inhibits the anti-tumor effect of natural killer cells and $CD8^+$ T cells by blocking their action[22–24]. Elevated expression of both the membrane-bound and soluble forms of MICA (sMICA) have been reported in several cancers, including HCC[25–27]. Exon 5 of *MICA* encodes the transmembrane domain and contains a variable number of tandem repeats (VNTR) consisting of 4, 5, 6 or 9 repeats of GCT or one additional G nucleotide insertion into the 5-GCT-repeat allele (referred as A4, A5, A6, A9 and A5.1, respectively). The insertion of G (A5.1) causes a premature stop codon and subsequent loss of the transmembrane domain, leading to altered subcellular localization[28]. Therefore, we tested whether rs2596542 is in linkage with functional *MICA* VNTR alleles.

We further genotyped 673 cases with HCV-induced HCC and 890 non-HCV controls for the *MICA* VNTR locus with capillary-based electrophoresis (**Supplementary Fig. 7**). A case-control analysis revealed that the *MICA* VNTR is associated with HCV-induced HCC (global $P = 4.55 \times 10^{-7}$; **Supplementary Table 11**). Particularly, alleles A9 and A6 were associated with conferring a higher risk of HCC (OR = 1.73 and OR = 1.34, respectively), whereas the A5 and A5.1 alleles had a protective effect. Comparison of the genotypes at rs2596542 and the VNTR locus revealed that the A risk allele at rs2596542 is in LD with the A9 and A4 alleles, and the non-risk G allele is in LD with the A5 and A5.1 alleles, whereas we observed no linkage between an A6 allele and rs2596542 (**Supplementary Table 12**). We also genotyped 124 individuals with CHC; however, we observed no significant association between individuals with CHC and controls or individuals with CHC and HCC (**Supplementary Tables 13,14**).



We then tested whether the VNTR alleles, rs2596542 alleles, or VNTR-rs2596542 haplotypes had any association with MICA expression in individuals with HCV-induced HCC. We determined sMICA levels by ELISA using a total of 665 HCC serum samples (**Supplementary Table 15**). Notably, rs2596542 was significantly correlated with sMICA levels, and specifically, the risk genotype AA was associated with low levels of sMICA ($P = 1.38 \times 10^{-13}$; **Fig. 2**), whereas VNTR alleles (**Supplementary Fig. 8**) and VNTR- rs2596542 haplotypes (**Supplementary Table 16**) showed no strong association. The absence of any correlation between *MICA* VNTR alleles and sMICA suggests that sMICA levels are not regulated by post-translational processing or a premature stop codon caused by A5.1 alleles in individuals with HCC. We also examined the sMICA level in different stages of HCV-induced liver disease (in non-HCV subjects and those with CHC and HCV-induced liver cirrhosis) and found that sMICA level was elevated at the early stage of disease and was not correlated with disease progression (**Fig. 3**). Additionally, the risk allele A was also correlated with low sMICA levels in subjects with CHC (**Supplementary Fig. 9**). These findings suggest that MICA expression was induced by factors caused by chronic HCV infection,

Figure 3 Correlation between soluble MICA and HCV-related diseases. The *x* axis shows the disease stages after HCV infection, and the *y* axis shows the concentration of soluble MICA in pg/ml. The number of independent samples tested in each group is shown in parentheses. Each group is shown as a box plot, and the median values are shown as thick dark horizontal lines (median values of non-HCV = 0, CHC = 64.55, LC = 72.11 and HCC = 77.98). The box covers the twenty-fifth to seventy-fifth percentiles, and the whiskers outside the box extend to the highest and lowest value within 1.5 times the interquartile range. Points outside the whiskers are outliers. We tested the difference in the median values among the disease groups using the Wilcoxon rank test. The box plots were plotted using default settings in R. Non-HCV, individuals not exposed to HCV infection; CHC, individuals with chronic hepatitis C; LC, individuals with liver cirrhosis; HCC, individuals with hepatocellular carcinoma.

similar to various types of stresses such as viral infection, inflammation and heat shock[29,30]. The levels of sMICA were shown to be directly proportional to the level of membrane-bound MICA[25], and membrane bound MICA is essential for activating natural killer cells and $CD8^+$ T cells to eliminate virus-infected cells[19]. Considering the association of the risk allele A with low levels of sMICA, our findings suggest that the individuals who carry the rs2596542 A allele would express low levels of membrane-bound MICA in response to HCV infection, which thus leads to poor or no activation of natural killer cells and $CD8^+$ T cells against virus-infected cells. Eventually, these individuals are likely to progress from CHC to HCC. Notably, several SNPs that are in absolute linkage with rs2596542 are located within the promoter or enhancer region of *MICA* and may alter the binding of stress-inducible transcriptions factors such as heat shock proteins (**Supplementary Table 17**). In this regard, it is important to analyze the factors that regulate *MICA* expression, particularly in the context of CHC. Although, the molecular mechanism whereby *MICA* polymorphisms confer the risk of disease progression should be characterized in the future, our findings reveal a crucial role of genetic variations in the host innate immune system in the development of HCV-induced HCC.

URLs. R, http://cran.r-project.org/; PLINK, http://pngu.mgh.harvard.edu/~purcell/plink/; Primer3 v0.3.0, http://frodo.wi.mit.edu/primer3/; LocusZoom, http://csg.sph.umich.edu/locuszoom/; FastSNP, http://fastsnp.ibms.sinica.edu.tw/pages/input_CandidateGeneSearch.jsp.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

K.M. and Y.N. conceived of the study; Y.N., V.K., M.K. and K.M. designed the study; V.K., Y.U., R.M. and N.H. performed genotyping; V.K., Y.N. and K.M. wrote the manuscript; A.T. and N. Kamatani performed quality control at the genome-wide phase; Y.N., K.M., H.N. and M.K. managed DNA and serum samples belonging to BioBank Japan; N. Kato, R.T., M. Otsuka, M. Omata and K.K. managed replication DNA and serum samples; V.K. analyzed the data, performed VNTR genotyping, ELISA and summarized the whole results; Y.N. obtained funding for the study.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Sample collections. We obtained DNA from 721 HCV-related HCC cases, 1,730 CHC cases and 5,486 HCV-negative controls from the BioBank Japan project[31]. For replication analysis, DNA from 673 HCV-induced HCC cases was obtained from a prospective HCC study cohort of the University of Tokyo. A diagnosis of CHC, liver cirrhosis or HCC were based on histological, clinical and laboratory findings obtained by trained physicians. Case samples with HBV co-infection were excluded from the analysis. Interferon was administrated to 20.4% of HCC cases and 70.1% of cases were not treated. The remaining 9.5% of the cases lacked information about interferon treatment. The non-HCV controls obtained from BioBank Japan contained case-mixed individuals after excluding all individuals with cancer, chronic hepatitis B, diabetes or tuberculosis. All subjects were of Japanese origin and provided written informed consent. The clinical and demographic details of the samples are summarized in **Supplementary Table 1**. We also obtained serum samples from BioBank Japan and the University of Tokyo (**Supplementary Table 12**). This research project was approved by the ethical committees of the University of Tokyo and RIKEN.

SNP genotyping and quality control. In the GWAS, 721 individuals with HCV-related liver cancer and 2,890 controls were genotyped using Illumina HumanHap610-Quad and Illumina HumanHap550v3 Genotyping BeadChip, respectively. In the replication stage, 673 cases with HCV-related disease, 1,730 cases with CHC and 2,596 controls were genotyped by the multiplex PCR-based Invader assay (Third Wave Technologies) and the Illumina HumanHap610-Quad, respectively. The common SNPs between the Illumina HumanHap550v3 and the Illumina HumanHap610-Quad arrays from all autosomal chromosomes were included for the analysis. We applied standard SNP quality control filters to exclude SNPs with low call rate (<99%), a Hardy-Weinberg equilibrium $P < 1.0 \times 10^{-6}$ for controls and minor allele frequency of <0.01. In the end, we obtained 432,703 SNPs for the analysis. In the replication analysis, the allele discrimination plots were validated by two well-trained researchers (the plots are available on request). We excluded samples with low genotyping rate (<99%) and employed principal component analysis to avoid the population stratification issue, in which individuals belonging only to Hondo cluster were included in the analysis (**Supplementary Fig. 10**)[32].

Statistical analysis. The association of SNPs with the disease phenotype in the GWAS, replication stage and combination analyses was tested using multivariate logistic regression analysis after adjusting for age at recruitment (continuous) and gender by assuming an additive model and using PLINK[33]. In the GWAS, the genetic inflation factor (λ) was derived by applying logistic regressed P values for all the tested SNPs. The quantile-quantile plot was drawn using R. The ORs were calculated by considering the major allele as a reference, unless it was stated otherwise elsewhere. The combined analysis of the GWAS and replication stage was verified by conducting the Mantel-Haenszel method. We considered $P < 5 \times 10^{-8}$ as the genome-wide significance threshold, which is the Bonferroni-corrected threshold for the number of independent SNPs genotyped in HapMap Phase 2 (ref. 34). Heterogeneity across the two stages was examined by using the Breslow-Day test[35].

For multiple logistic regression analysis at rs2596542 using the R program, we considered age at recruitment (≤60 or >60 years)[3], gender (male or female) and alcohol consumption (non-drinkers, ≤ 50 g alcohol per day or >50 g alcohol per day) as covariates from both the GWAS and replication stage cases with HCC and non-HCV controls. Association at the *MICA* VNTR locus was analyzed by Fisher's exact test, and the global P value was calculated using a χ^2 test. Statistical comparisons between genotypes and sMICA levels were performed by Kruskal-Wallis test or Wilcoxon rank test using R. We employed the R package haplo.stats to infer haplotypes and to perform haplotype association analysis. P values for association between sMICA levels and haplotype distribution were obtained by score test under an additive model by using the haplo.score function. ORs and 95% confidence intervals were calculated from the coefficients of the GLM model by considering the major haplotype as a reference. We used the haplo.cc function to calculate these statistical values.

HCV serotype. HCV serotype data was available for 531 cases with HCC from the replication stage. HCV serotype was examined by serotyping assay (SRL Laboratory) according to previously reported methods[36]. According to the Simmonds classification[37], serotype 1 corresponded to disease types 1a and 1b, whereas serotype 2 corresponded to disease types 2a and 2b.

***MICA* VNTR locus genotyping.** We followed the method suggested by Applied Biosystems. Briefly, the 5′ end of the forward primer was labeled with 6-FAM, and the 5′ end of reverse primer was labeled with the GTGTCTT non-random sequence to promote addition of As. The primer sequences were previously reported[28]. The PCR products were mixed with Hi-Di Formamide and GeneScan-600 LIZ size standard and separated using a GeneScan system on a 3730xl DNA analyzer (Applied Biosystems). GeneMapper software (Applied Biosystems) was used to assign the repeat fragment size (**Supplementary Fig. 7**).

Quantification of soluble MICA. sMICA levels were measured by sandwich enzyme-linked immunosorbent assay, as described in the manufacturer's instructions (R&D Systems).

Imputation and association analysis at HLA allele tagging SNPs. We obtained a SNP or a combination of SNPs which can tag HLA alleles in the Japanese population from a previous study[13]. The untyped genotypes of these SNPs were imputed in the GWAS samples by using a hidden Markov model programmed in MACH[38] and haplotype information from HapMap JPT samples. We applied the same SNP quality criteria as in the GWAS for selecting SNPs for the analysis. The association was tested on all SNPs that passed the quality control criteria using logistic regression analysis conditioned on age and gender.

Initially, we obtained the pair-wise LD between HLA alleles tagging SNPs and rs2596542. We performed case-control association analysis in our GWAS dataset. As shown in **Supplementary Table 9**, none of the HLA-tagging SNPs showed evidence of linkage or association except rs2844521, and rs2844521 was in absolute linkage with rs2596542 ($r^2 = 1$, $D' = 1$) and thus showed similar association. We obtained actual genotype data at rs2596501, as this SNP is included on the 550K SNP platform, and inferred the haplotype between rs2844521 and rs2596501. However, the haplotype GT (the G allele of rs2844521 and the T allele of rs2596501), which is reported to tag the *HLA-B*3501* allele ($r^2 = 1$, $D' = 1$), was not associated with HCC in our GWAS dataset ($P = 0.39$). We also performed a conditional logistic regression analysis on rs2596501 (data not shown) and found no effect on the association between rs2596542 and HCV-induced HCC. This data suggested that rs2596542 association is independent of *HLA-B*3501*. Although we observed mild association between other *HLA-B* alleles (*HLA-B*5401*, $P = 0.004$; *HLA-B*6701*, $P = 0.012$) and HCV-induced HCC, the association at rs2596542 alone was the most significant. Taken together, we found no strong evidence for linkage of HLA alleles with rs2596542.

Software. For general statistical analysis, we used R statistical environment version 2.6.1 or plink version 1.06. The Haploview software version 4.2 (ref. 39) was used to calculate LD and to draw Manhattan plots. Primer3 v0.3.0 web tool was used to design primers. We used LocusZoom for plotting regional association plots. We used FastSNP[40] web tool for functional annotation of SNPs (see URLs for all software packages).

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Research Article

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Telaprevir with peginterferon and ribavirin for treatment-naive patients chronically infected with HCV of genotype 1 in Japan

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Background & Aims: To evaluate the efficacy and safety of telaprevir in combination with peginterferon-α2b (PEG-IFN) and ribavirin (RBV) in patients with chronic hepatitis C.

Methods: In a multi-center randomized clinical trial in Japan, on patients infected with HCV of genotype 1, 126 patients were assigned to telaprevir for 12 weeks along with PEG-IFN and RBV for 24 weeks (Group A), while 63 to PEG-IFN and RBV for 48 weeks (Group B).

Results: HCV RNA disappeared more swiftly in patients in Group A than B, and the frequency of patients without detectable HCV RNA at week 4 (rapid virological response (RVR)) was higher in Group A than B (84.0% *vs.* 4.8%, p <0.0001). Grade 3 and 4 skin disorders, including Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms, as well as Grade 3 anemia (<8.0 g/dl), occurred more frequently in Group A than B (skin disorders, 11.9% *vs.* 4.8%; anemia, 11.1% *vs.* 0.0%). The total RBV dose was smaller in Group A than B (47.0% *vs.* 77.7% of the target, p <0.0001). Despite these drawbacks, sustained virological response (SVR) was achieved more frequently in Group A than B (73.0% *vs.* 49.2%, p = 0.0020).

Conclusions: Although the triple therapy with telaprevir-based regimen for 24 weeks resulted in more adverse events and less total RBV dose than PEG-IFN and RBV for 48 weeks, it was able to achieve higher SVR within shorter duration by carefully monitoring adverse events and modifying the RBV dose as required.

Keywords: Telaprevir; Chronic hepatitis C; Peginterferon; Ribavirin; Sustained virological response; Genotypes.

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Abbreviations: PEG-IFN, peginterferon; RBV, ribavirin; SVR, sustained virological response; SOC, standard of care; DAA, direct acting antiviral.

Introduction

Over the world, an estimated 170 million people are persistently infected with hepatitis C virus (HCV) [1]. Most individuals with persistent HCV infection can fulfill the life expectancy, while about 30% of them develop life-threatening liver disease such as decompensated cirrhosis and hepatocellular carcinoma [2,3].

Currently, interferon (IFN) is the only antiviral drug capable of terminating HCV infection. The present standard-of-care (SOC) therapy for patients infected with HCV of genotype 1, the most prevalent genotype over the world, is peginterferon (PEG-IFN) combined with ribavirin (RBV) for 48 weeks. However, sustained virological response (SVR), judged by the loss of detectable HCV RNA from serum 24 weeks after the completion of therapy, can be achieved in only 42–52% of the patients [4–6]. To cope with this grim situation, a number of direct acting antivirals (DAAs) have been designed and developed, represented by NS3/4A protease inhibitors and NS5B polymerase or NS5A inhibitors [7]. Among them, telaprevir has shown promising results, when combined with PEG-IFN and RBV, in the phase 2 [8,9] and 3 clinical trials [10,11], by improving SVR to ~70% in patients infected with HCV-1.

Previous trials with the triple therapy were conducted in Europe and the United States, respectively. Hence, Asians were under-represented, accounting only for 1.6–2.1% of studied patients, and distributions of genotypes 1a (44–67%) and 1b (27–55%) varied widely [8–10]. In view of ethnic differences in response to IFN-based treatments [12,13], as well as profiles of resistance to telaprevir difference between genotypes 1a and 1b [14], a multi-center, randomized, and treatment-controlled clinical trial was conducted for comparison of therapeutic efficacy between the triple therapy and SOC in patients infected with HCV-1b in Japan.

Patients and methods

Patients

From November 2008 through August 2010, 220 patients, who were infected with HCV-1 and had not received antiviral treatments before, were recruited at 41 institutions in Japan. They joined the study for finding differences in the

Table 1. Baseline characteristics of patients.

Features[a]	Group A: T12PR24 (n = 126)	Group B: PR48 (n = 63)
Men (%)	66 (52.4%)	33 (52.4%)
Age (years)	53.0 (20-65)	55.0 (20-65)
Weight (kg)	60.2 (40.7-87.5)	64.1 (42.1-84.9)
BMI (kg/m^2)	22.6 (16.2-31.1)	23.3 (17.9-30.8)
Hemoglobin (g/dl)	14.3 (12.1-17.1)	14.5 (12.3-17.5)
White blood cells (/mm^3)	5300 (2900-10,670)	5130 (2950-11,050)
Platelets (x10^4/mm^3)	19.2 (9.0-36.2)	20.2 (8.7-37.0)
ALT (IU/L)	36.5 (12-252)	45.0 (18-259)
AST (IU/L)	34.0 (18-170)	38.0 (17-142)
Total bilirubin (mg/dl)	0.70 (0.3-1.9)	0.80 (0.4-1.8)
Total cholesterol (mg/dl)	182 (111-299)	180 (116-263)
HCV RNA ($\log_{10}$ IU/ml)	6.7 (5.1-7.5)	6.9 (5.1-7.4)
HCV genotypes		
1a	2 (1.6%)	0 (0.0%)
1b	124 (98.4%)	63 (100.0%)

[a]Values are the median with the range in parentheses, or number with the percentage in parentheses.

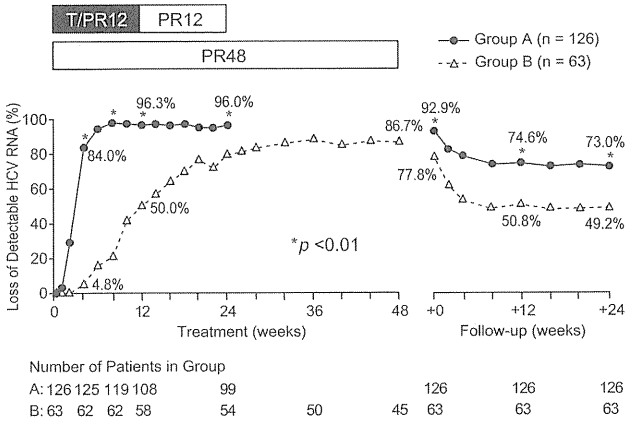

Fig. 1. Loss of detectable HCV RNA in patients in Groups A and B. Statistical tests were performed at weeks 4, 8, 12, and 24 in the treatment period, end of treatment, and weeks 12 and 24 in the follow-up period. An asterisk (*) indicates p <0.01 differences. The number of patients at each time point is indicated below the graph.

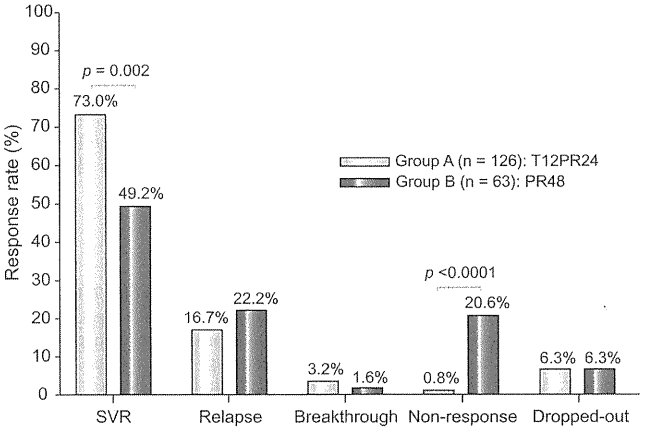

Fig. 2. Comparison of treatment responses between patients in Groups A and B. SVR, sustained virological response (HCV RNA negative 24 weeks after the completion of treatment); relapse, reappearance of HCV RNA in serum during follow-up period; breakthrough, reappearance of HCV RNA in serum during treatment period; non-response, HCV RNA continuously detectable in serum during treatment period.

treatment response and adverse events between the triple therapy involving telaprevir, PEG-IFN and RBV, and SOC with PEG-IFN and RBV. The study protocol complied with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the review board of each institution. Each patient gave a written informed consent before participating in this study.

Study design

This prospective, multi-center, and randomized study was planned on Japanese patients with chronic hepatitis C who met inclusion and did not meet exclusion criteria. Main inclusion criteria were: (a) diagnosed with chronic hepatitis C, and had not received antiviral treatments before; (b) infected with HCV-1 confirmed by the sequence analysis in the NS5B region; (c) had HCV RNA levels $\geqslant$5.0 $\log_{10}$ IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics K.K. Tokyo, Japan); (d) Japanese aged from 20 to 65 years at the entry; (e) had the body weight between >40 and $\leqslant$120 kg; (f) were not pregnant and capable of contraception till 24 weeks after the treatment; and (g) agreed on the admission for 15 days since the treatment start. Main exclusion criteria were: (a) decompensated liver cirrhosis; (b) hepatitis B surface antigen; (c) hepatocellular carcinoma or other malignancy, or its history; (d) autoimmune hepatitis, alcoholic liver disease, hemochromatosis or chronic liver disease other than chronic hepatitis C; (e) depression or schizophrenia, or its history, or history of suicide attempts; (f) chronic renal disease or creatinine clearance $\leqslant$50 ml/min at the baseline; (g) hemoglobin <12 g/dl, neutrophil counts <1500/mm^3 or platelet counts <100,000/mm^3 at the baseline; and (h) pregnancy in progress or planned during the study period of either partner.

Patients were randomly assigned to either of the following two treatment groups in a 2:1 ratio, with stratification to balance sex and age: (1) the triple therapy with telaprevir, PEG-IFN, and RBV for 12 weeks, followed by PEG-IFN and RBV for an additional 12 weeks (Group A: T12PR24); and (2) SOC with PEG-IFN and RBV for 48 weeks (Group B: PR48). After the treatment was completed or discontinued, they were followed for $\geqslant$24 weeks for SVR evaluation. Patients were followed regularly for subjective symptoms and objective signs, as well as blood

Table 2. Comparison of SVR stratified by demographic and virological factors as well as discontinuation of study drugs between two groups with different therapeutic regimens.

	A: T12PR24 n = 126	B: PR48 n = 63	Differences *p* value
Gender			
Men	50/66 (75.8%)	18/33 (54.5%)	0.0400
Women	42/60 (70.0%)	13/30 (43.3%)	0.0214
Age (years)			
≤49	35/41 (85.4%)	13/21 (61.9%)	0.0543
≥50	57/85 (67.1%)	18/42 (42.9%)	0.0125
HCV RNA (log_{10} IU/ml)			
≥7	18/26 (69.2%)	5/18 (27.8%)	0.0132
<7	74/100 (74.0%)	26/45 (57.8%)	0.0556
Discontinuation of study drugs			
Not discontinued	66/79 (83.5%)	27/46 (58.7%)	0.0030
All drugs discontinued	14/27 (51.9%)	4/17 (23.5%)	0.1143

counts and chemistry. HCV RNA levels were monitored at day −28, days 1 (pre-dose), 2, and 3, weeks 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 (both groups), as well as weeks 26, 28, 32, 36, 40, and 48 (Group B), during the treatment period; they were monitored at weeks 2, 4, 8, 12, 16, 20, and 24 in the follow-up period (both groups).

HCV RNA and genotypes

HCV RNA was quantified using the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). The linear dynamic range of this assay was 1.2–7.8 log_{10} IU/ml, and samples with no HCV RNA detected were reported as: <1.2 log_{10} IU/ml (no HCV RNA detectable). Genotypes of HCV were determined by direct sequencing followed by phylogenetic analysis of the NS5B region [15].

Antiviral treatments

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) 750 mg was administered three times a day at an 8-h interval (q8h) after each meal. Peginterferon-α2b (PegIntron®, MSD, Tokyo, Japan) was injected subcutaneously at a median dose of 1.5 μg/kg (range: 1.250–1.739 μg/kg) once a week. Ribavirin (Rebetol®, MSD, Tokyo, Japan) 200–600 mg was administered after breakfast and dinner. The daily dose of RBV was adjusted to the body weight: 600 mg for ≤60 kg; 800 mg for >60 kg ~≤80 kg; and 1000 mg for >80 kg.

RBV dose was diminished by 200 mg in patients receiving 600 or 800 mg (by 400 mg in those receiving 1000 mg) when hemoglobin decreased <12 g/dl, and by extra 200 mg when it lowered <10 g/dl. In addition, RBV was reduced by 200 mg in patients with hemoglobin <13 g/dl at baseline or in those in whom it decreased by 1 g/dl within a week and below 13 g/dl. Dose modification of RBV in Group B was conducted in accordance with SOC. PEG-IFN dose was reduced to one half, when leukocyte counts decreased <1500/mm^3, neutrophil counts <750/mm^3 or platelet counts <8 × 10^4/mm^3; PEG-IFN was discontinued when they decreased <1000/mm^3, 500/mm^3 or 5 × 10^4/mm^3, respectively. The triple therapy was discontinued or interrupted when hemoglobin decreased <8.5 g/dl. In patients whose hemoglobin increased ≥8.5 g/dl within 2 weeks after the interruption, treatment was resumed with PEG-IFN and RBV 200 mg. The reduction of telaprevir dose was not permitted.

Statistical analysis

SVR was evaluated in the full analysis set. The difference in SVR between Groups A and B with the 2-sided 95% confidence interval (CI) was calculated with the adjustment for sex and age, and *p* value was evaluated by the Wald-test. Continuous variables between groups were compared by the Mann–Whitney test (*U*-test), and categorical variables by the Fisher's exact test. Statistical analyses were performed using the statistical software SAS Version 9.1 (SAS Institute Inc., Cary, NC), and a *p* value <0.05 was considered significant.

Results

Patient cohorts

Of the 220 Japanese patients from whom an informed consent was obtained, 31 (14.1%) were found not eligible for the study entry. The remaining 189 patients were randomly assigned to T12PR24 (Group A [n = 126]) or PR48 (Group B [n = 63]). Overall, 114 out of the 126 (90.0%) patients in Group A and 54 out of the 63 (85.7%) in Group B completed the full study period. Table 1 compares baseline characteristics of studied patients in Groups A and B. There were no differences in demographic characters, hematology, biochemistry, or virology between the two groups of patients.

Loss of HCV RNA during treatment

Dynamics of HCV RNA during treatment was much different between Groups A and B. HCV RNA disappeared more frequently (98.4% *vs.* 79.4%, p <0.001) and swiftly (within 8 *vs.* 38 weeks) in patients in Group A than B. Time courses of the loss of HCV RNA are compared in Fig. 1. The loss of HCV RNA increased constantly, sharply, and swiftly in Group A. By contrast, in Group B, it gradually increased during the first 24 weeks of treatment. Rapid virological response at 4 weeks (RVR) occurred more frequently in Group A than B (84.0% *vs.* 4.8%, p <0.0001). HCV RNA was undetectable in >90% of patients in Group A, while it stayed undetectable in <80% of patients in Group B at the start of follow-up. After treatment completion, HCV RNA re-appeared in patients in both Groups A and B (16.7% *vs.* 22.2%, p = 0.4272).

Responses to treatments

Fig. 2 compares treatment responses between Groups A and B. SVR was achieved more frequently in Group A than B (73.0% *vs.* 49.2%, p = 0.0020). By contrast, non-response was less frequent in Group A than B (0.8% *vs.* 20.6%, p <0.0001). The difference in SVR between Groups A and B, adjusted for sex and age, was 23.8% (95% CI: 9.4–38.2%, p = 0.0012, Wald-test).

Table 3. Adverse events developing in more than 15% of patients in either Groups A or B.

	A: T12PR24 (n = 126)		B: PR48 (n = 63)	
Anemia	115	(91.3%)	46	(73.0%)
Pyrexia	98	(77.8%)	46	(73.0%)
Leukocytopenia	86	(68.3%)	46	(73.0%)
Thrombocytopenia	81	(64.3%)	23	(36.5%)
Malaise	73	(57.9%)	30	(47.6%)
Serum uric acid increased	65	(51.6%)	5	(7.9%)
Serum hyaluronic acid increased	64	(50.8%)	25	(39.7%)
Alopecia	51	(40.5%)	29	(46.0%)
Headache	48	(38.1%)	32	(50.8%)
Skin rashes	48	(38.1%)	18	(28.6%)
Anorexia	42	(33.3%)	17	(27.0%)
Insomnia	40	(31.7%)	17	(27.0%)
Vomiting	37	(29.4%)	9	(14.3%)
Drug eruption	37	(29.4%)	2	(3.2%)
Arthralgia	36	(28.6%)	15	(23.8%)
Serum triglycerides increased	36	(28.6%)	11	(17.5%)
Dysgeusia	34	(27.0%)	10	(15.9%)
Diarrhoea	34	(27.0%)	19	(30.2%)
Nausea	32	(25.4%)	7	(11.1%)
Serum creatinine increased	32	(25.4%)	0	
Erythema at the injection site	33	(26.2%)	21	(33.3%)
Reactions at the injection site	29	(23.0%)	16	(25.4%)
Stomatitis	24	(19.0%)	12	(19.0%)
Abdominal discomfort	23	(18.3%)	12	(19.0%)
Pruritus	23	(18.3%)	13	(20.6%)
Nasopharyngitis	23	(18.3%)	18	(28.6%)
Influenza-like symptoms	22	(17.5%)	16	(25.4%)
Serum bilirubin increased	22	(17.5%)	13	(20.6%)
Back pain	21	(16.7%)	12	(19.0%)
Hyperuricemia	20	(15.9%)	2	(3.2%)
Serum phosphorus decreased	16	(12.7%)	13	(20.6%)
Constipation	14	(11.1%)	13	(20.6%)
Erythema	9	(7.1%)	13	(20.6%)

Factors influencing the treatment response are compared in Table 2. SVR was higher in Group A than B, irrespective of different genders, age ranges, or HCV RNA loads. Of note, SVR in women in Group A was higher than that in Group B (70.0% *vs.* 43.3%, $p = 0.0214$). Likewise, SVR in patients $\geqslant 50$ years was higher in Group A than B (67.1% *vs.* 42.9%, $p = 0.0125$), and that in patients with high HCV RNA loads ($\geqslant 7$ $\log_{10}$ IU/ml) at the baseline was higher in Group A than B (69.2% *vs.* 27.8%, $p = 0.0132$).

Adverse events

Adverse events occurred in all patients in both Groups A and B. Adverse events with a frequency >15% in either group are listed in Table 3. Of them, frequencies of anemia, thrombocytopenia, malaise, and elevated serum levels of uric acid as well as hyaluronic acid were >10% higher in Group A than B. Most of them were mild, and severe and serious adverse events occurred in small proportions of patients (9.5% and 11.9% in Group A, respectively, and 9.5% and 9.5% in Group B). All drugs were discontinued due to adverse events comparatively frequently in Groups A and B (16.7% and 22.2%, respectively), and telaprevir alone in 19.0% of patients in Group A. The total dose of RBV was less in Group A than B (47.0% *vs.* 77.7% of the target, $p < 0.0001$). Doses of antiviral treatments were reduced or discontinued in some patients with moderate to severe adverse events, patients were taken care of by specialists, and received specific therapies when necessary. Eventually, all patients recovered from adverse events.

Hematological disorders

Anemia occurred in 91.3% and 73.0% of patients in Groups A and B, respectively. Table 4 compares the severity of anemia between Groups A and B. Combined, Grade 1 and 2 anemia developed more frequently in Group A than B (38.1% *vs.* 17.5%, $p = 0.0045$). Grade 3 anemia occurred in 11.1% in Group A only. During the follow-up, hemoglobin increased both in Groups A and B, and returned to pretreatment levels 12 weeks after the completion of therapy and thereafter (Fig. 3A). Platelet counts decreased more extensively in Group A than B (Fig. 3B). They rebounded after the completion of therapy, and then returned to pretreatment values. Decreases in neutrophil counts were milder in Group A than B (Fig. 3C). Both in Groups A and B, neutrophils started to increase immediately after the treatment completion, and returned to pretreatment levels within 12 weeks.

Skin disorders

Skin disorders were monitored at every hospital visit for severity and extent, and they were categorized into four Grades (Table 4). When skin disorders of Grades 2–4 occurred, the attendant physician was instructed to consult with a dermatologist in each institution for the diagnosis and specific cares, and telaprevir was discontinued, while PEG-IFN and RBV were reduced or discontinued, as required. Skin disorders were mainly rash, drug eruptions, and erythema. They occurred comparably frequently in Groups A and B (89.7% and 84.1%, respectively). Most skin disorders were mild and categorized into Grade 1 in 75.4% and 76.2% of patients in Groups A and B, respectively. Combined, skin disorders of Grades 2–4 occurred more frequently in Group A than B (46.8% *vs.* 23.8%, $p = 0.0026$). Due to skin disorders, at least one drug was discontinued in merely 9.5% and 3.2% of patients in Groups A and B, respectively, and most skin disorders were controllable by anti-histamine and/or steroid ointments.

Serious skin disorders developed in three patients in Group A, but none in Group B. Stevens–Johnson syndrome occurred in one patient 35 days after the treatment start, and led to the discontinuation of treatment. Erythema spread widely in the trunk (Fig. 4A), as well as limbs and the face. Erosion of oral mucosae, epidermal detachment, conjunctival redness, high fever to reach 39.3 °C, and lymphadenopathy were also noted. Histopathology showed the epidermal necrosis, satellite-cell necrosis, and perivascular dermatitis with infiltration of lymphocytes, neutrophils, and eosinophils in the superficial dermis (Fig. 4B). The patient was admitted and received steroids intravenously, and recovered completely within 9 weeks. Drug rash with eosinophilia and

Table 4. Decreases in hemoglobin levels and skin disorders according to the grade.

Grade	A: T12PR24 n = 126	B: PR48 n = 63	Differences *p* value
A Hemoglobin levels			
Grade 1 (9.5- <11.0 g/dl)	50 (39.7%)	32 (50.8%)	0.1631
Grade 2 (8.0- <9.5 g/dl)	34 (27.0%)	11 (17.5%)	0.2043
Grade 3 (<8.0 g/dl)	14 (11.1%)	0	0.0055
Total	98 (77.8%)	43 (68.3%)	0.1613
B Skin disorders			
Grade 1[a]	95 (75.4%)	48 (76.2%)	1.0000
Grade 2[b]	44 (34.9%)	12 (19.0%)	0.0282
Grade 3[c]	13 (10.3%)	3 (4.8%)	0.2709
Grade 4[d]	2 (1.6%)	0 (0.0%)	0.5532
Any grade	113 (89.7%)	53 (84.1%)	0.3451

[a]Localized skin lesions.
[b]Diffuse or multiple skin lesions.
[c]Skin lesions covering >50% of the body surface or rashes with some characteristics such as bullae, ulceration of mucous membrane, epidermal detachment, target lesion or significant systemic signs.
[d]Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms (DRESS) were categorized in Grade 4.

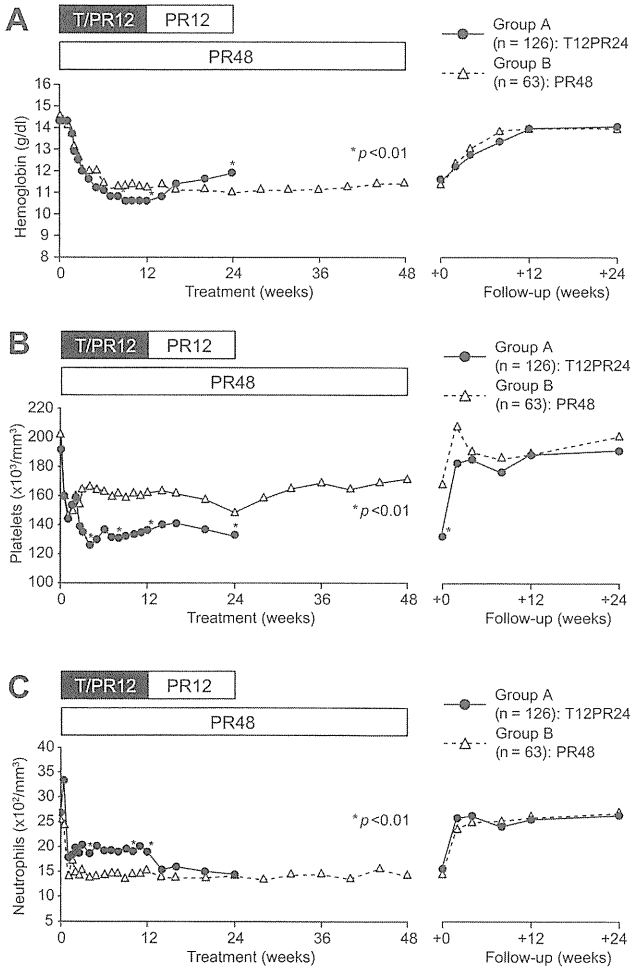

Fig. 3. Comparison of hematopoietic disorders between patients in Groups A and B. (A) Median hemoglobin levels, (B) platelet counts, and (C) neutrophil counts are plotted during treatment and follow-up. Ranges from 25% to 75% are omitted for visual clarity. Statistical tests were performed at weeks 4, 8, 12, and 24 in the treatment period, end of treatment, and at weeks 12 and 24 in the follow-up period. An asterisk (*) indicates p <0.01 difference.

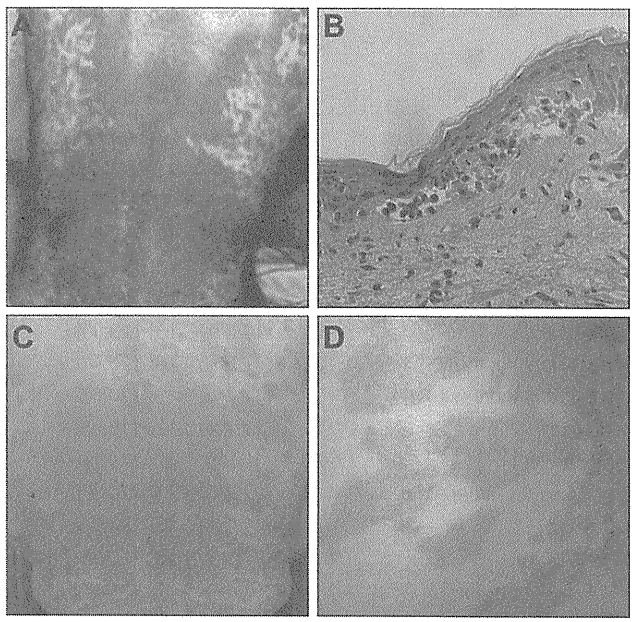

Fig. 4. Grade 4 skin regions in patients who received the triple therapy. (A) Erythema and (B) histopathology of the skin in the patient with Stevens–Johnson syndrome, as well as (C and D) generalized erythema in the patient developing drug rashes with eosinophilia and systemic symptoms (DRESS), are shown.

systemic symptoms (DRESS or drug-induced hypersensitivity syndrome) occurred in another patient. Fresh red erythema appeared on the whole body, and fresh red-colored target lesions (up to 3–4 cm in diameter) were also observed (Fig. 4C and D). Edema in the face, lymphadenopathy, fever up to 39.7 °C, and erosion of oral mucosae were noted, also. Maximum levels of white blood cells, eosinophils, and atypical lymphocytes were 46,300/mm^3, 45.7%, and 23.3%, respectively. Titers of IgG antibody to human herpes virus 6 were ×160 (29 days after the onset) and ×2560 (57 days). The remaining patient developed erythema multiforme. These two patients received steroids orally and recovered completely within 14 weeks.

Discussion

A prospective, randomized, and treatment-controlled clinical trial was planned and conducted in Japan to compare the therapeutic efficacy and safety profiles between the triple therapy with T12PR24 and the SOC treatment with PR48. In this trial, 126 patients were assigned to receive T12PR24 (Group A) and the 63 to receive PR48 (Group B). They all were treatment-naive, and infected with HCV-1 in high viral loads ($\geqslant 5$ $\log_{10}$ IU/ml) and of genotype 1b in the great majority (98.9%). Randomization was not adopted due to ethnical concerns against giving intravenous placebo weekly for 24 weeks to patients in Group A.

Dynamics of circulating HCV RNA during treatment was quite different between Groups A and B. HCV RNA disappeared more frequently (98.4% *vs.* 79.4%, p <0.001) and swiftly (within 8 *vs.* 38 weeks) in patients in Group A than B. Accordingly, SVR was achieved more frequently in patients with T12PR24 than PR48 (73.0% *vs.* 49.2%, $p = 0.0020$), while rates of relapse (16.7% *vs.* 22.2%) and breakthrough (3.2% and 1.6%) were not different between them. Due to the higher therapeutic efficacy and shorter treatment duration, T12PR24 would be more suitable for treatment of HCV-1 patients than the standard PR48, and lessen the total economic burden of patients and the nation.

Previous clinical trials with telaprevir were conducted in Europe or the United States and combined with PEG-IFN-α2a [8–11]. In the present study, Japanese patients have responded to a triple therapy with PEG-IFN-α2b, with an efficacy of 73% in comparison with 72–75% in phase 3 clinical trials [10,11]. In a recent report, PEG-IFN-α2a and -α2b were equally effective in triple therapies in combination with telaprevir and RBV [16]. Frequency of side effects demanding the discontinuation of all drugs is comparable between patients receiving the triple therapy with PEG-IFN-α2a in phase 3 trials [10,11] and -α2b in the present study (7–17% and 17%, respectively).

In our previous report [17], the IFN-responsive C/C genotype of *IL28B* at rs12979860 was detected in 42 out of the 72 (55%) patients infected with HCV-1 in Japan; the prevalence was not much different from that in 336 out of the 769 (44%) European-Americans [18]. The susceptibility to telaprevir depends on HCV genotypes, and is higher for genotypes 1 and 2 than genotypes 4 and 5 in *in vitro* experiments [19]. Further, it may differ between 1a and 1b, due to dissimilar evolution patterns of drug-resistant mutations [14]. Nevertheless, present patients infected with HCV-1b in the great majority (98.4%) were equally responsive to the triple therapy with telaprevir as those infected with HCV-1a [8,9,11].

High efficacy of T12PR24 was accompanied by increased adverse events, of which anemia and skin lesions were worrisome. Moderate and severe anemia (<9.5 g/dl) developed more frequently in Group A than B (38.1% *vs.* 17.5%, $p = 0.0045$). Since Japanese patients with chronic hepatitis C are older by >10 years than those in Western countries, with a higher proportion of women, they are prone to develop anemia during treatment with telaprevir. Stringent precaution had to be taken, therefore, by deducting the RBV dose in patients in whom hemoglobin levels decrease <12 g/dl, higher than the conventional threshold of <10 g/dl. The total RBV dose was lower in Group A than B (47.0% *vs.* 77.7% of the target, p <0.0001). However, decreased doses of RBV or PEG-IFN did not influence substantially the therapeutic efficacy of T12PR24.

Skin disorders of Grades 2–4 occurred more frequently in Group A than B (46.8% *vs.* 23.8%, $p = 0.0026$). It has to be noted that Grade 4 skin lesions, such as Stevens–Johnson syndrome and drug rashes with eosinophilia and systemic symptoms (DRESS), developed exclusively in patients in Group A. Since studied patients were monitored carefully and received immediate care by dermatologists, if and when skin lesions of Grades 2–4 developed, all patients eventually recovered. In the area of DAAs, potentially accompanying severe skin disorders, physicians would need close cooperation with dermatologists for the care of patients with hepatitis C.

In conclusion, this multicenter, randomized, and treatment-controlled study of T12PR24 in Japanese patients infected with HCV-1b has proven the efficacy and safety comparable to those in previous phase 3 studies [10,11]. Due to the excellence of T12PR24 over the standard PR48, we hope it will be used widely in patients with chronic hepatitis C over the world, who are expected to increase rapidly in the foreseeable future [20].

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Efficacy and safety of telaprevir, a new protease inhibitor, for difficult-to-treat patients with genotype 1 chronic hepatitis C

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SUMMARY. The aims of this phase III study were to assess the efficacy and safety of telaprevir in combination with peginterferon alfa-2b (PEG-IFN) and ribavirin (RBV) for difficult-to-treat patients who had not achieved sustained virological response (SVR) to prior regimens in Japan. The subjects were 109 relapsers (median age of 57.0 years) and 32 nonresponders (median age of 57.5 years) with hepatitis C virus genotype 1. Patients received telaprevir (750 mg every 8 h) for 12 weeks and PEG-IFN/RBV for 24 weeks. The SVR rates for relapsers and nonresponders were 88.1% (96/109) and 34.4% (11/32), respectively. Specified dose modifications of RBV that differed from that for the standard of care were introduced to alleviate anaemia. RBV dose reductions were used for 139 of the 141 patients. The SVR rates for relapsers did not depend on RBV dose reduction for 20–100% of the planned dose (SVR rates 87.5–100%, $P < 0.05$). Skin disorders were observed in 82.3% (116/141). Most of the skin disorders were controllable by anti-histamine and/or steroid ointments. The ratios of discontinuation of telaprevir only or of all the study drugs because of adverse events were 21.3% (30/141) and 16.3% (23/141), respectively. A frequent adverse event leading to discontinuation was anaemia. Telaprevir in combination with PEG-IFN/RBV led to a high SVR rate for relapsers and may offer a potential new therapy for nonresponders even with a shorter treatment period.

Keywords: direct-acting antiviral, peginterferon, ribavirin, sustained virological response, treatment failure.

INTRODUCTION

Hepatitis C virus (HCV) affects approximately 170 million people worldwide [1]; patients with chronic hepatitis C (CHC) eventually develop cirrhosis and hepatocellular carcinoma (HCC) [2,3]. The standard of care (SOC) with peginterferon plus ribavirin (RBV) for 48 weeks is most effective for eradicating HCV genotype 1 [4], which is a dominant genotype for CHC [1]. However, the sustained virological response (SVR) rate of SOC for the treatment of naïve patients with genotype 1 is approximately <50% [5,6]. The retreatment regimen for patients who do not achieve SVR is limited to exposure to peginterferon plus RBV with modification of dose and treatment duration. Some studies have been conducted to estimate the effectiveness of peginterferon plus RBV for 48 weeks for nonresponders to prior interferon-based combination therapy, and the SVR rates in most studies did not exceed 20% [7–9]. A large randomized study of patients who had not responded to previous treatment with peginterferon alfa-2b (PEG-IFN) plus RBV gave SVR rates for peginterferon alfa-2a 180 μg/kg plus RBV for 72 weeks that were not as high as those for 48 weeks (14%, 9%) [10]. HCV patients who had failed to achieve SVR with the combination therapy displayed high risk rates of decompensated cirrhosis, HCC and liver-related mortality [11]. Therefore, it is very important to establish new regimens to increase the SVR rate and shorten the treatment period for patients who do not achieve SVR with prior treatments.

Telaprevir, classified as a direct-acting antiviral agent, is a reversible, selective, orally bioavailable inhibitor of the nonstructural NS3/4A HCV serine protease [12]. Two phase II studies (PROVE 1 and PROVE 2) on the treatment of naïve patients with genotype 1 were conducted to assess the

Abbreviations: CHC, chronic hepatitis C; ETR, end of treatment response; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PEG-IFN, peginterferon alfa-2b; RBV, ribavirin; RVR, rapid viral response; SOC, standard of care; SVR, sustained virological response.

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efficacy of telaprevir for 12 weeks in combination with peginterferon and RBV for 24 weeks [13,14]. These studies demonstrated that the SVR rates of the telaprevir regimen were significantly higher compared with SOC (PROVE 1: 61% *vs* 41%, $P = 0.02$, PROVE 2: 69% *vs* 46%, $P = 0.004$). A subsequent phase II study (PROVE 3) for treatment-failure patients with genotype 1 gave SVR rates for nonresponders, relapsers and breakthroughs in the telaprevir regimen of 39%, 69% and 57%, respectively [9].

In Japan, a phase III study was conducted for the treatment of naïve patients with genotype 1 to compare the efficacy and safety between the telaprevir regimen and SOC. It has demonstrated that the SVR rate for the telaprevir regimen was significantly higher than that for SOC (73.0% *vs* 49.2%, $P = 0.0020$) [15]. We decided to conduct a phase III study to assess the efficacy and safety of telaprevir in combination with PEG-IFN and RBV in relapsers and nonresponders who had not achieved SVR to a previously administered IFN-based regimen in Japan.

PATIENTS AND METHODS

Study patients

Relapsers and nonresponders were enrolled in Study 1 (ClinicalTrials.gov Identifier: NCT00780910) and Study 2 (ClinicalTrials.gov Identifier: NCT00781274), respectively. Relapsers were defined as patients who had been previously treated for CHC and had undetectable HCV RNA during interferon or peginterferon therapy (including combination with RBV). Nonresponders were defined as patients who were previously treated for CHC and had never had undetectable HCV RNA for more than 24 weeks with interferon or peginterferon therapy (including combination with RBV).

The patients were enrolled from 17 sites in Japan. Patients considered eligible were of 20–65 years of age, had CHC because of HCV genotype 1 (defined by NS5B sequence) [16] and ≥5.0 $\log_{10}$ IU/mL HCV RNA level at the screening test, had been previously treated for CHC with interferon or peginterferon therapy (including combination with RBV), had a body weight of 40 kg or more and below 120 kg, could be hospitalized for at least 2 weeks after the first administration, were not pregnant and agreed to contraception from the screening period to 24 weeks after the last dosing of the study drug. The patients were excluded if they had a haemoglobin level of <12 g/dL, neutrophil count of <1500/mm^3, platelet count of <100 000/mm^3, were positive for HBs antigen and HIV antibodies at the screening test, had chronic renal failure or creatinine clearance of ≤50 mL/min, depression, schizophrenia or its history, history of suicide attempt, decompensated cirrhosis, previous or current HCC or other malignancies, autoimmune hepatitis, alcoholic liver disease or haemochromatosis.

All patients provided written informed consent before participating in the study. These studies were approved by each site's institutional review board and conducted in accordance with good clinical practice and the Declaration of Helsinki.

Study design

All patients received PEG-IFN (PegIntron®; MSD, Tokyo, Japan) at a dose of 1.5 μg/kg per week subcutaneously, RBV (Rebetol®; MSD) at a dose of 600 mg per day (for body weight ≤60 kg), 800 mg per day (for body weight >60 to ≤80 kg) or 1000 mg per day (for body weight >80 kg) and telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) at a dose of 750 mg every 8 h after food. The patients were treated with telaprevir, PEG-IFN and RBV for 12 weeks, followed by PEG-IFN and RBV (PEG-IFN/RBV) for 12 weeks. All patients had a 24-week follow-up period after the last dosing of study drugs to assess SVR.

Dose modification of study drugs

Specified dose modification of RBV that differed from the dose for SOC was introduced to alleviate anaemia. The initial dose of RBV was reduced by 200 mg per day in case of a haemoglobin level <13 g/dL at baseline. The RBV dose was reduced by 200 mg per day in patients receiving 600 or 800 mg per day (by 400 mg per day in those receiving 1000 mg) when the haemoglobin level was <12 g/dL and was reduced by an additional 200 mg per day when the haemoglobin level was <10 g/dL. The RBV dose was also reduced by 200 mg per day if the haemoglobin level dropped ≥1 g/dL within 1 week, and this level was <13 g/dL. Telaprevir was withdrawn when the haemoglobin level was <8.5 g/dL. PEG-IFN/RBV were withdrawn or interrupted when the haemoglobin level was <8.5 g/dL. The dose modifications of PEG-IFN were followed by SOC. Dose modification and interruption of telaprevir were not allowed. Telaprevir was withdrawn if serious adverse events appeared. The use of erythropoietin was not allowed for elevating the haemoglobin level.

Stopping rules

Patients could be discontinued from the study at any time if the investigator or sponsor determined that it was not in the interest of the patient to continue the study or the patient wished to withdraw from the study. The study drugs were discontinued if the patients had a haemoglobin level of <8.5 g/dL, white blood cell count of <1000/mm^3, neutrophil count of <500/mm^3 or platelet count of <50 000/mm^3.

In case of the following criteria for serum HCV RNA viral kinetics measured during the treatment period, discontinuation of the study drugs was decided at the investigator's discretion. (i) When the following criteria applied twice consecutively: (a) the amount of change from the lowest value for HCV RNA level exceeded 2.0 $\log_{10}$ IU/mL and (b)

HCV RNA level exceeded 2.0 $\log_{10}$ IU/mL after it had been confirmed to be <1.2 $\log_{10}$ IU/mL. (ii) When the serum HCV RNA level at 13 weeks after administration of study drugs did not decrease by >2.0 $\log_{10}$ IU/mL from the baseline level.

Efficacy assessments

Serum HCV RNA levels were measured using the COBAS TaqMan HCV test (Roche Diagnostics Co. Ltd., Tokyo, Japan). The linear dynamic range was 1.2–7.8 $\log_{10}$ IU/mL. Samples with undetectable HCV RNA were reported as '<1.2 $\log_{10}$ IU/mL (no detectable HCV RNA)'. Measurements were obtained at week 4 before day 1 of the screening period: at days 1 (predose), 2 and 3; weeks 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 of the treatment period; and weeks 2, 4, 8, 12, 16, 20 and 24 of the follow-up period.

The primary endpoint was a SVR defined as an undetectable HCV RNA level 24 weeks after the end of treatment. Relapse, breakthrough, and nonresponse were defined based on AASLD Guidelines as follows [4]: 'relapse' was a state of undetectable serum HCV RNA at the end of treatment and reappearance of serum HCV RNA during the follow-up period; 'breakthrough' was a state of undetectable serum HCV RNA and reappearance of serum HCV RNA during the treatment period; and 'nonresponse' was a state of continuously detectable serum HCV RNA during the treatment period.

Safety assessments

All adverse events were recorded up to the last visit and coded using MedDRA/J version 13.0. (MedDRA Japanese Maintenance Organization, Tokyo, Japan) Measurements for chemical laboratory data were obtained at week 4 before day 1 of the screening period: at day 1 (predose); weeks 1, 2, 4, 8, 10, 12, 14, 16, 18, 20 and 24 of the treatment period; and weeks 2, 4, 8, 12 and 24 of the follow-up period. Electrocardiogram (ECG) and fundus examinations were performed once during the screening period. Adverse events, haematological and chemical laboratory data, and vital signs were assessed and summarized. The severity of rash was categorized into three grades.

Statistical analysis

Sustained virological response rates were evaluated for the full analysis set. Categorical variables were compared by Fisher's exact test. Statistical analyses were performed using the statistical software SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA), and a P value < 0.05 was considered significant.

Table 1 Baseline characteristics of study patients

	Study 1 (relapsers) $N = 109$	Study 2 (nonresponders) $N = 32$
Gender – *n* (%)		
Men	66 (60.6)	17 (53.1)
Women	43 (39.4)	15 (46.9)
Age, years – median (range)	57.0 (20, 65)	57.5 (40, 65)
Weight, kg – median (range)	62.50 (41.0, 92.5)	61.30 (44.9, 92.5)
BMI, kg/m^2 – median (range)*	23.10 (18.0, 32.4)	22.60 (17.1, 31.2)
ALT (IU/L) – median (range)†	36.0 (16, 302)	48.0 (17, 190)
Haemoglobin (g/dL) – median (range)	14.70 (12.0, 17.8)	14.50 (12.3, 16.6)
White blood cell count ($/mm^3$)	4680.0 (2490, 15940)	4830.0 (3040, 8000)
Platelet count ($\times 10^4/mm^3$) – median (range)	17.80 (9.9, 33.8)	17.85 (9.1, 26.2)
HCV RNA ($\log_{10}$ IU/mL) – median (range)‡	6.75 (5.2, 7.6)	6.78 (6.0, 7.7)
HCV genotype 1 subtype – *n* (%)		
1a	0 (0.0)	1 (3.1)
1b	109 (100.0)	31 (96.9)
Prior therapy for chronic hepatitis C – *n* (%)		
Interferon	13 (11.9)	1 (3.1)
Interferon plus ribavirin	14 (12.8)	2 (6.3)
Peginterferon	3 (2.8)	0 (0.0)
Peginterferon plus ribavirin	79 (72.5)	29 (90.6)

HCV, hepatitis C virus.
*The body mass index (BMI) is the weight in kilograms divided by the square of the height in metres; †Alanine aminotransferase; ‡The HCV RNA level was measured using the COBAS TaqMan HCV test (Roche).

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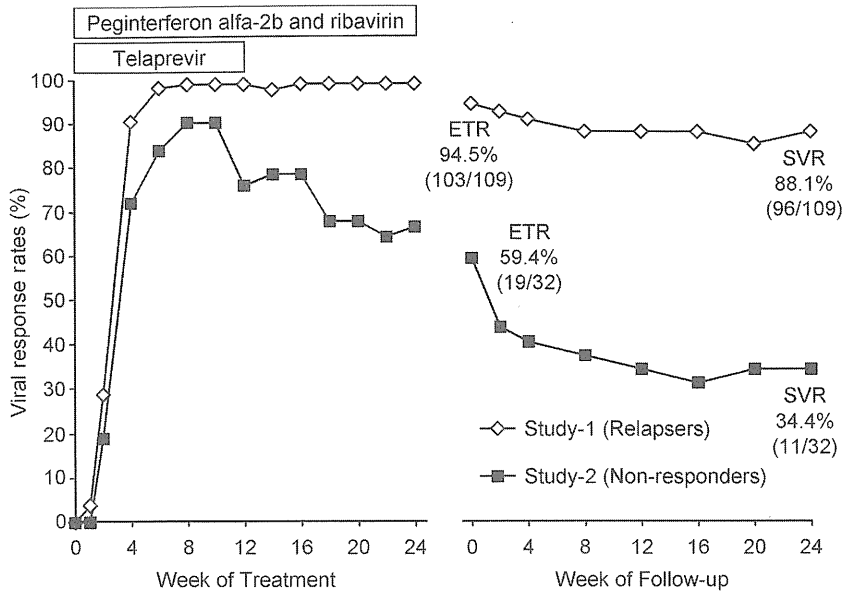

Fig. 1 Undetectable hepatitis C virus RNA rates at each measurement point. SVR, sustained virological response; ETR, end-of-treatment response.

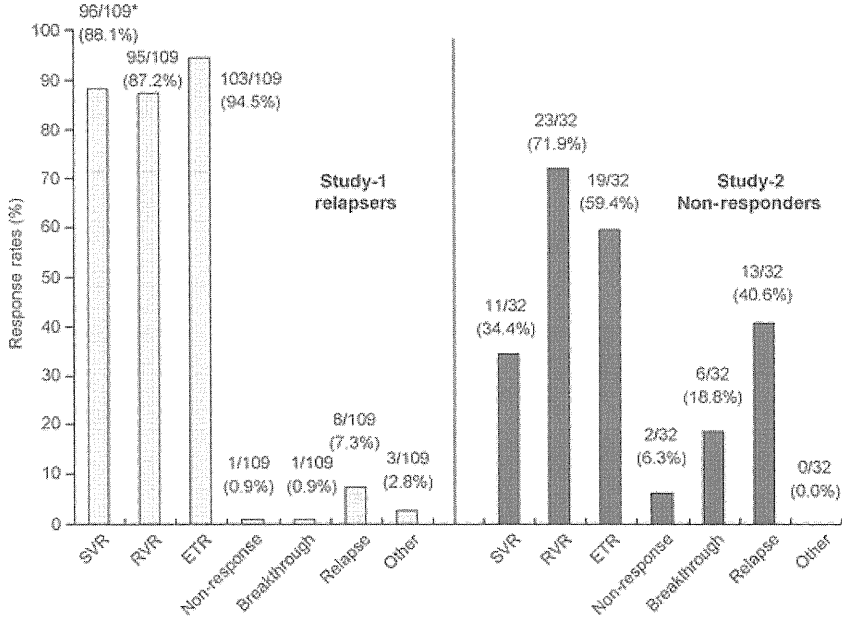

Fig. 2 Response rates of patients with virological response. *Number of patients who achieved SVR in each subgroup/*N* (%). SVR, sustained virological response; RVR, rapid viral response; ETR, end-of-treatment response.

RESULTS

Study patients

From November 2008 to August 2009, a total of 168 patients [Study 1 (*N* = 135) and Study 2 (*N* = 33)] were screened, and 141 patients [Study 1 (*N* = 109) and Study 2 (*N* = 32)] received at least one dose of a study drug. The baseline characteristics of the study patients are shown in Table 1. Patients previously treated with PEG-IFN (with or without RBV) and IFN (with or without RBV) in Study 1 and Study 2 accounted for 75.2% (82 of 109) and 24.7% (27 of 109) and 90.6% (29 of 32) and 9.4% (3 of 32), respectively. The median of age, weight, haemoglobin level, platelet count and HCV RNA level for Study 1 and Study 2 were 57.0 and 57.5 years, 62.5 and 61.3 kg, 14.7 and 14.5 g/dL, 17.8

and $17.85 \times 10^4/mm^3$, and 6.75 and 6.78 log_{10} IU/mL, respectively. Patients over 50 years of age accounted for 81.7% (89 of 109) and 81.3% (26 of 32), respectively.

Efficacy in study 1 (relapsers)

Figure 1 shows the change in the undetectable HCV RNA rates at each measurement point. The rapid viral response (RVR) rate and the end of treatment response (ETR) rate were 87.2% (95/109) and 94.5% (103/109), respectively. The SVR rate, nonresponse, breakthrough and relapse were 88.1% (96/109), 0.9% (1/109), 0.9% (1/109) and 7.3% (8/109), respectively (Fig. 2).

Factors influencing the SVR rate are compared in Table 2. The SVR rate in the patients who achieved undetectable HCV RNA at ≤week 4 was significantly higher than that in the patients who achieved undetectable HCV RNA at >week 4 (91.8% *vs* 66.7%, $P = 0.0487$). Also, the SVR rate for men was significantly higher than that for women (93.9% *vs* 79.1%, $P = 0.0316$). The SVR rate with discontinuation of all the study drugs was significantly lower than that with discontinuation of only telaprevir or no discontinuation of the study drugs (all the study drugs: 60.0%, only telaprevir: 95.0% and no discontinuation: 94.2%, $P = 0.0007$). In contrast, there was no difference in the SVR rate in relation to HCV RNA level and prior therapy for CHC. SVR rates by the ratio of the actual total RBV dose to the anticipated total RBV dose were evaluated (Fig. 3). The SVR rates did not depend on RBV dose reduction for 20–100% of the planned dose (87.5–100%, $P < 0.05$).

Efficacy in study 2 (nonresponders)

The RVR and ETR rates were 71.9% (23/32) and 59.4% (19/32), respectively (Fig. 1). The SVR rate, nonresponse, breakthrough and relapse were 34.4% (11/32), 6.3% (2/32), 18.8% (6/32) and 40.6% (13/32), respectively (Fig. 2). There was no difference in the SVR rate in relation to

Table 2 SVR rates stratified by demographic, undetectable HCV RNA and discontinuation of study drug treatment

	Study 1 (relapsers) $N = 109$	Study 2 (nonresponders) $N = 32$
Gender – *n/N* (%)		
Male	62/66 (93.9)	8/17 (47.1)
Female	34/43 (79.1)	3/15 (20.0)
P-value	0.0316	0.1475
Age – *n/N* (%)		
≤49	18/20 (90.0)	2/6 (33.3)
≥50	78/89 (87.6)	9/26 (34.6)
P-value	1.0000	1.0000
HCV RNA (log_{10} IU/mL) – *n/N* (%)		
≥7.0	26/30 (86.7)	5/10 (50.0)
<7.0	70/79 (88.6)	6/22 (27.3)
P-value	0.7498	0.2515
Prior therapy for chronic hepatitis C – *n/N* (%)		
Interferon	12/13 (92.3)	1/1 (100.0)
Interferon plus ribavirin	13/14 (92.9)	2/2 (100.0)
Peginterferon	3/3 (100.0)	– (–)
Peginterferon plus ribavirin	68/79 (86.1)	8/29 (27.6)
P-value	0.9271	0.0333
Undetectable – *n/N* (%)		
≤Week 4	90/98 (91.8)	9/23 (39.1)
>Week 4 ≤end of treatment	6/9 (66.7)	2/7 (28.6)
P-value	0.0487	1.0000
Discontinuation of study drug treatment – *n/N* (%)		
No discontinuation	65/69 (94.2)	9/20 (45.0)
Telaprevir only	19/20 (95.0)	2/7 (28.6)
All study drugs	12/20 (60.0)	0/5 (0.0)
P-value	0.0007	0.1711

SVR, sustained virological response; HCV, hepatitis C virus.
SVR was defined as an undetectable HCV RNA level 24 weeks after the end of treatment.

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baseline characteristics, HCV RNA level and prior treatment for CHC. The SVR rates for the patients who received 40–80% RBV dose reduction were over 30% (Fig. 3).

Safety

Adverse events were observed in all the patients in Study 1 and Study 2. Adverse events observed in at least 15% of the patients in each clinical study are listed in Table 3. Adverse events were similar between Study 1 and Study 2. Most of the adverse events were mild and moderate. Serious adverse events in Study 1 and Study 2 were reported in 11.9% (13/109) and 9.4% (3/32) of the patients, respectively. The ratios of discontinuation of all the study drugs because of adverse events in Study 1 and Study 2 were 17.4% (19/109) and 12.5% (4/32), respectively. A frequent adverse event leading to discontinuation was anaemia. Discontinuation rates of all the study drugs because of anaemia in Study 1 and Study 2 were 10.1% (11/109) and 9.4% (3/32), respectively. One death was reported in Study 1. One patient in Study 1 died of pulmonary embolism. Causality of PEG-IFN and RBV was classified as 'probably related' and that of telaprevir was classified as 'possibly related'.

Adverse events related to skin disorders were observed in 82.3% (116/141) of the patients. Skin disorders reported in over 10% of the patients were rash in 39.0% (55/141), drug eruption in 24.1% (34/141), injection site reaction in 12.8% (18/141) and injection site erythema in 12.8% (18/141) of the patients. Most of the skin disorders were controllable by anti-histamine and/or steroid ointments. Grade 3 (severe) skin disorders in Study 1 and Study 2 were reported in 6.4% (7/109) and 6.3% (2/32) of the patients, respectively. Discontinuation of all the study drugs because of skin disorders in Study 1 amounted to 3.7% (4/109). No discontinuation because of skin disorders occurred in Study 2.

Figure 4 shows the changes in haemoglobin levels, platelet counts and neutrophil counts during the treatment and follow-up periods. Changes in the haematological parameters were similar between Study 1 and Study 2. The platelet count and neutrophil count decreased sharply within 4 weeks and then gradually decreased. Despite the modification of RBV, the median haemoglobin levels in Study 1 and Study 2 decreased to 10.6 and 10.4 g/dL at week 12, respectively. No patient discontinued all the study drugs because of neutrophil decrease. The haematological parameters recovered to the baseline level at the end of the follow-up period.

DISCUSSION

This phase III study was planned and conducted to assess the efficacy and safety of telaprevir in combination with PEG-IFN/RBV for relapsers and nonresponders. Most of the patients who participated in this study had received a prior PEG-IFN/RBV regimen. Despite a shorter treatment period, the SVR rates for relapsers and nonresponders were 88.1% and 34.4%, respectively. The result indicates that the HCV RNA response to previous treatment history should be one of the diagnostic factors for predicting SVR.

The SVR rate for men was significantly higher than that for women in the relapser group (93.9% *vs* 79.1%, $P = 0.0316$). There was no significant difference in other characteristics of the patients in that group. Once the relapsers had achieved undetectable HCV RNA, this condi-

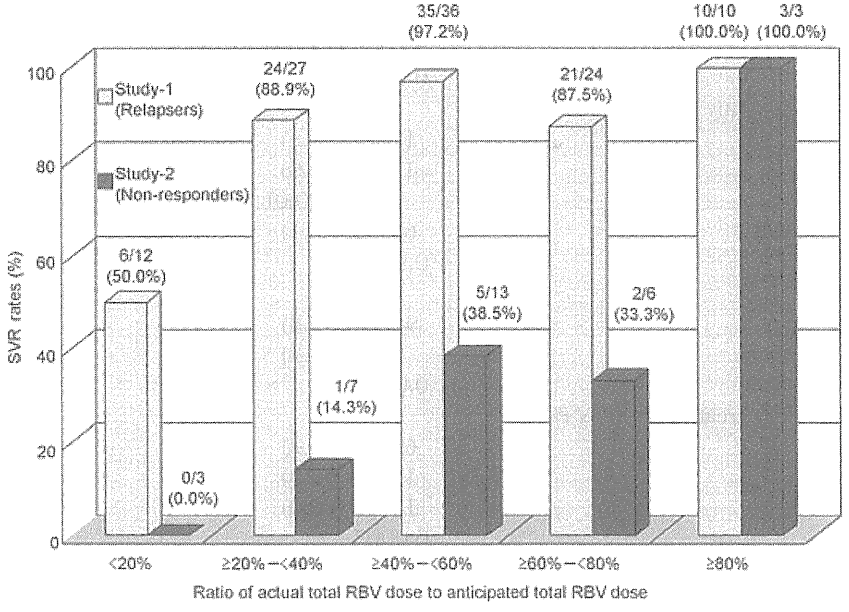

Fig. 3 Sustained virological response rates according to adherence to the ribavirin dose.

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Table 3 Most common adverse events

MedDRA/J (Version.13.0) preferred term – *n* (%)	Study 1 (relapsers) *N* = 109	Study 2 (nonresponders) *N* = 32	Total *N* = 141
Anaemia	96 (88.1)	32 (100.0)	128 (90.8)
Pyrexia	90 (82.6)	30 (93.8)	120 (85.1)
White blood cell count decreased	83 (76.1)	22 (68.8)	105 (74.5)
Blood uric acid increased	72 (66.1)	25 (78.1)	97 (68.8)
Platelet count decreased	73 (67.0)	22 (68.8)	95 (67.4)
Malaise	60 (55.0)	23 (71.9)	83 (58.9)
Decreased appetite	56 (51.4)	15 (46.9)	71 (50.4)
Hyaluronic acid increased	56 (51.4)	15 (46.9)	71 (50.4)
Rash	39 (35.8)	16 (50.0)	55 (39.0)
Headache	42 (38.5)	10 (31.3)	52 (36.9)
Blood creatinine increased	36 (33.0)	12 (37.5)	48 (34.0)
Insomnia	34 (31.2)	11 (34.4)	45 (31.9)
Blood bilirubin increased	34 (31.2)	10 (31.3)	44 (31.2)
Alopecia	35 (32.1)	7 (21.9)	42 (29.8)
Diarrhoea	31 (28.4)	7 (21.9)	38 (27.0)
Dysgeusia	29 (26.6)	6 (18.8)	35 (24.8)
Vomiting	26 (23.9)	8 (25.0)	34 (24.1)
Drug eruption	24 (22.0)	10 (31.3)	34 (24.1)
Nausea	24 (22.0)	4 (12.5)	28 (19.9)
Abdominal discomfort	22 (20.2)	6 (18.8)	28 (19.9)
Blood triglycerides increased	19 (17.4)	8 (25.0)	27 (19.1)
Pruritus	20 (18.3)	2 (6.3)	22 (15.6)
Arthralgia	18 (16.5)	4 (12.5)	22 (15.6)
Nasopharyngitis	19 (17.4)	2 (6.3)	21 (14.9)
Stomatitis	13 (11.9)	6 (18.8)	19 (13.5)
Back pain	12 (11.0)	5 (15.6)	17 (12.1)
Blood phosphorus decreased	10 (9.2)	6 (18.8)	16 (11.3)

The adverse events listed are those that were reported in at least 15% of patients in each clinical study.

tion was sustained until the end of the treatment period. The patients who achieved RVR had a higher SVR rate than the patients who had no RVR in the relapser group (91.8% *vs* 66.7%, $P = 0.0487$).

In contrast, there was no significant difference related to characteristics in the nonresponder group. The SVR rates between men and women and undetectable HCV RNA were, however, slightly different. As Study 2 for the nonresponders was of a small scale, it will be necessary to evaluate a larger number of patients. The breakthrough ratio in the nonresponders during the PEG-IFN/RBV treatment period and relapse ratio were 18.8% and 40.6%, respectively. Two patients were nonresponders with high telaprevir-resistant variants; one was subtype 1a and the only patient with this characteristic in the study.

Triple therapy for 12 weeks, followed by PEG-IFN/RBV for 12 weeks for the relapsers led to a high SVR rate. In contrast to the relapsers, all breakthroughs were observed in 18.8% of nonresponder patients after the end of telaprevir treatment, and relapse were observed in 40.6% of nonresponder patients after the end of treatment period. Continuation of telaprevir over 12 weeks and PEG-IFN/RBV over 24 weeks might be needed to achieve a higher SVR rate for nonresponders.

Dose modification of RBV that differed from that for SOC was introduced to prevent anaemia in the patients [17]. Dose reductions of RBV were observed in 98.6% of the patients, and those who had 200 mg RBV per day as a minimum dose and those who discontinued it accounted for 41.8% and 29.8%, respectively. The haemoglobin level recovered to the baseline level at the end of the follow-up period. As a result of dose modification, the change in the haemoglobin level in this study was similar to that in PROVE 3 [9]. Checking the haemoglobin level once a week during the treatment period is important. The SVR rates did not depend on RBV dose reduction among the relapsers who had over 20% of the anticipated total RBV dose (87.5–100%). Thus, it is important to monitor haemoglobin levels and continue RBV dosing appropriately to achieve SVR, even with a low RBV dose.

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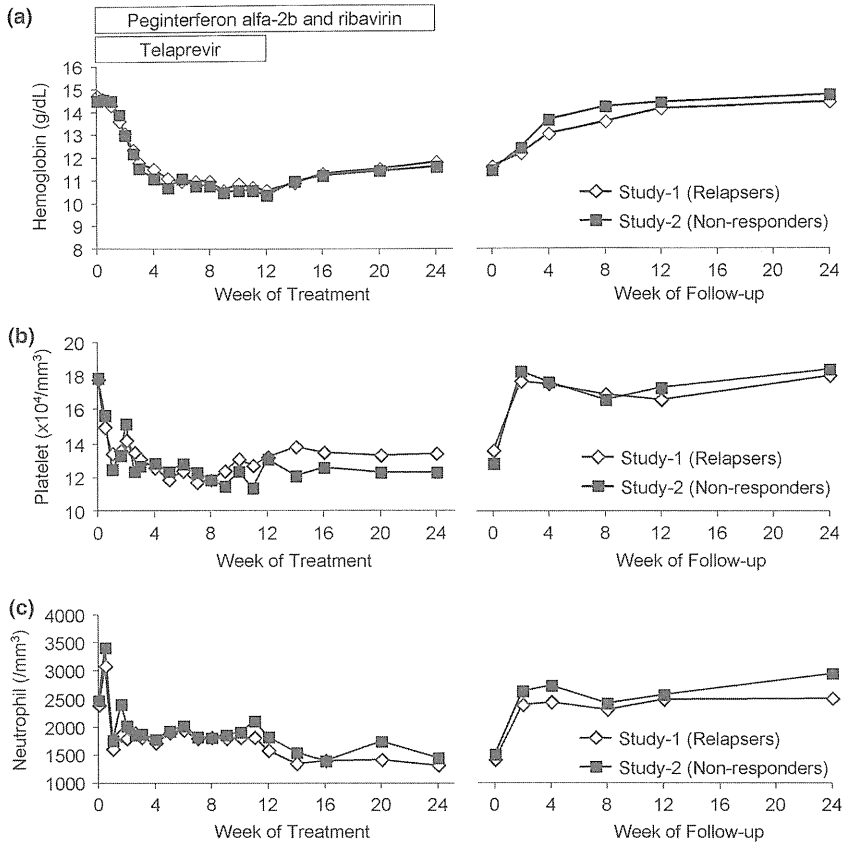

Fig. 4 Changes in hematology parameters. Median haemoglobin levels (a), median platelet counts (b) and median neutrophil counts (c) were plotted during treatment and follow-up periods.

Adverse events related to skin disorder were reported by 82.3% of the subjects. Of the nine cases of severe skin disorders, seven occurred within 8 weeks. Telaprevir was likely to be related to the occurrence of the severe skin disorders. The mechanism of skin disorders is unknown. All the patients who discontinued treatment received immediate care from dermatologists and recovered eventually. Skin disorders should be carefully monitored by physicians in collaboration with dermatologists.

The relationship between the SVR rates and the difference in SNPs in gene IL28B or near IL28B has become clear [18,19]. With genetic variation in rs8099917, SVR rates of 83.8% and 27.6% were achieved for patients with genotype TT and non-TT who were treated with telaprevir in combination with PEG-IFN/RBV, respectively [20]. Also, genetic variations in gene ITPA related to haemoglobin decrease and reduction of RBV has been discussed for patients treated with PEG-IFN/RBV [21,22]. We did not evaluate IL28B and ITPA in this study. As anaemia was the most frequent adverse event leading to the discontinuation of the study drugs in the present study, it should become a valuable pharmacogenetic diagnostic tool to optimize the triple therapy.

In conclusion, this phase III study conducted in Japan demonstrated that telaprevir in combination with PEG-IFN/RBV had a high SVR rate for relapsers and shows promise as a potential therapy for nonresponders even with a short treatment period. Prolongation of telaprevir and PEG-IFN/RBV treatment should be a better option for achieving high SVR for nonresponders. As the data demonstrated convincingly that the benefits greatly outweigh the risks, telaprevir-based regimen is at the lead for the next generation of HCV therapies.

DISCLOSURES

None to declare.

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