by oligo ligation and detection". It uses a unique sequencing method catalyzed by DNA ligase. The universal P1 adaptor-linked DNA fragments are attached to magnetic beads. Emulsion PCR is conducted in microreactors containing the reagents of the PCR reaction. The magnetic beads are covalently attached to the surface of a specially treated glass slide that is placed into a fluidic cassette within the sequencer. The universal sequence primers hybridize to the P1 adapter within the library template. The set of four fluorescent-labeled di-base 8-mer probes are annealed to the sequencing primer and library template. Identification of the nucleotide sequence by the 8-mer probe is achieved by interrogating every first and second base in each ligation reaction. When there is a matching of the 8-mer probe to the library template adjacent to the universal primer of the 3′-end, DNA ligase seals the phosphate backbone. After the ligation, the probe is enzymatically removed together with the last three bases attaching the linkage between base 5 and 6. Then, the same probe hybridizing process is conducted and the sequence data of each library template can be obtained at five nucleotide intervals. Following a series of ligation cycles, the library template is reset with five rounds of universal primers complementary to the n to n-4 position for a multistep round of ligation cycles. Through the primer reset process, each base is interrogated in two independent ligation reactions by two different primers and the nucleotide sequence is defined by this repetition.

The ABI SOLiD 2.0 platform, produced in 2008, can obtain data output from 3–10 Gb per run.[16–18]

THIRD- AND FOURTH-GENERATION NGS SYSTEM

HOWEVER, MUCH IMPROVED the NGS systems have already become, the competition in technology development is intensifying. The demand for low cost, high speed and highly accurate systems has spurred development beyond third-generation NGS systems (Table 1).

Ion semiconductor sequencing was introduced by Ion Torrent Systems (South San Francisco, CA, USA) and was released in 2010. The principle of the technology is based on detecting hydrogen ions released in the reaction-induced changes of the pH of the solution by an ion sensor when the nucleotide base is incorporated by DNA polymerase. Its read length of approximately 100 bp is comparable to that of other NGS systems, but the throughput is still lower, although increasing the size of the semiconductor chips could improve the throughput.[19,20]

Pacific Biosciences (PacBio, Menlo Park, CA, USA) developed a single molecule real-time sequencer based on single molecule real-time sequencing by the synthesis method with monitoring of the deoxyribonucleotide triphosphate (dNTP) uptake of DNA sequencing by DNA polymerase. The fluorescently labeled dNTP is incorporated and the fluorescent dye is separated from the DNA. The sequencing reaction is conducted on zero-mode wave guides (ZMW) that are small well-containers with detectors located at the bottom of the well. The detectors can capture the fluorescent dye. The

Table 1 The classification of NGS systems

Classification	Second-generation	Third-generation	Fourth-generation
Principal	Using "sequencing-by-synthesis" method by DNA polymerase or DNA ligase, the nucleotide sequence is determined in parallel by optical detection such as fluorescence emission	By carrying out the DNA synthesis by DNA polymerase referring as one template DNA molecule, the nucleotide sequence is determined in real time by detecting fluorescence emission with one base sequencing reaction	The nucleotide sequence is determined by nanopore sequencing or by a method without the detection of fluorescent or luminescence emission
Throughput	1–50 Gy/day	45–60 Mb/run	>100 Mb/run (Ion Torrent)
Accuracy	Excellent	Error rate is relatively large	Excellent
Read length	25–700 bases	1–2 kilobases	100–200 bases (Ion Torrent)
Sequencer	Roche454 Illumina sequencers ABI SOLiD	PacBio Genia	Ion Torrent systems Oxford Nanopore

Table 2 Classification of next-generation sequencing (NGS) system by application

	Application	NGS system	Features
Research use	Advanced research application	PacBio RS Oxford Nanopore GridION	Single-molecule real-time sequencing, detection of DNA methylation and long-read sequencing
	General genome research application	Illumina HiSeq/Genome Analyzer IIx ABI SOLiD Roche 454 GS Ion Torrent Proton	Whole-genome sequencing with high throughput. Advanced knowledge of molecular biology is necessary for sequencing analysis
Clinical use	Clinical diagnosis application	Illumina MiSeq Ion Torrent PGM Oxford Nanopore MinION	Desktop type or USB memory stick type. Automatic extraction of DNA from samples or sequencing analysis

DNA polymerase is immobilized by only one molecule at the bottom. After the single template DNA is bound to the polymerase with incorporation of the fluorescently labeled dNTP, the DNA synthesis is performed. The DNA sequencing is conducted by detecting the separated fluorescent dye.[21] In January 2011, a paper from PacBio was published in the *New England Journal of Medicine* demonstrating the origin of the 2010 cholera outbreak in Haiti.[22] The PacBio RS was commercially released in early 2011 and had the advantage of a short time from equipping the library to sequencing, obtaining long reads and fewer errors or bias with PCR amplification. However, there is the disadvantage of low yield at high accuracy and low throughput.

Nanopore sequencing technology has been developing since 1995 for determining the sequence without nucleotide labeling and detection.[23] In brief, DNA sequencing with nanopore technology relies on the conversion of the electrical signals of nucleotides by passing through a nanopore, which is a specific protein pore covalently attached to the molecules. This approach is the most advanced and was demonstrated by Oxford Nanopore Technologies (Oxford Science Park, Oxford, UK).[24] Two nanopore sequencer models, the GridION sequencer which can perform large-scale sequencing, and the MinION sequencer, which is a portable and disposable sequencer, are planned for release. The MinION sequencer is a breakthrough device that overturns the concept of previous sequencers. The size of this sequencer is almost the same as a Universal Serial Bus (USB) memory stick and, after plugging this sequencer into the USB port of a personal computer, sequencing can be performed just by loading the sample. So far, this nanopore sequencer has tremendously surpassed other NGS systems. But there is a problem in that the error rate is still high compared with the Illumina or SOLiD sequencers.

Genia Technologies is a venture company founded in Mountain View city (CA, USA) in March 2009. They are now planning to launch the Genia sequencer in 2013. Genia technology combines the complementary metal–oxide–semiconductor (CMOS) chip technology of Ion Torrent and the nanopore sequencing by Stefan Roever.

The race to develop NGS systems is being carried out with the goal of "lower cost and higher performance". Therefore, we cannot select a sequencer in any appropriate analysis. We classified the three types of NGS systems for different applications. Type 1 (advanced research application) includes sequencers such as the PacBio RS or Oxford nanopore GridION, which can detect DNA methylation and perform long-read sequencing. Type 2 (general genome research application) includes sequencers such as the Illumina sequencer series or ABI SOLiD or Ion Torrent sequencers, which can be used for whole-genome sequencing with high throughput. Advanced knowledge of molecular biology is necessary for sequencing analysis. Type 3 (clinical diagnosis application) includes the Nanopore MinION, which can automatically conduct the extraction DNA from samples and the sequencing analysis (Table 2).

NGS APPLICATIONS TO VIRAL HEPATITIS

SINCE THE INTRODUCTION of the NGS sequencer in 2005, the production of large numbers of sequence reads made useful for many applications concerned with human genomes research, particularly whole-genome resequencing, de novo genome sequencing or transcriptomes (RNA-seq), genomic variation and mutation detection, genome-wide profiling of epigenetic marks and chromatin structure using ChIP-seq.

Currently, the identification of viral genome sequences is mainly cloning by PCR amplification with Sanger direct sequencing. Usually, viruses infecting a host have genomic diversity, referred to as "quasispecies". However, with this method it is difficult to measure the frequencies of each mutation, and it is impossible to detect several mutations combined in the same sequence. As an alternative to Sanger direct sequencing, molecular cloning can analyze single viral DNA molecules. However, this methodology is complicated and time-consuming. These complications can now be overcome by NGS technology. Therefore, this technology is suitable for whole viral genome sequencing, metagenomics, the identification of viral variants and viral dynamics. Some of the topics related to the clinical application for hepatitis virus will be described.

Hepatitis C virus (HCV)

The appearance of HCV variants is generated because of the high replication rate and the error-prone nature of RNA-dependent RNA polymerase. The selection of the mutants has developed to escape immunological and therapeutic control.[25] Moreover, the presence of contaminating nucleic acids of the host cell and other viral agents make it difficult to sequence the full-length HCV genome. In fact, the preparation of a library of cDNA synthesized from RNA with random priming results in a huge amount of host-specific genomes instead of the viral sequences, even in the presence of a very high viral load. High throughput sequencing techniques could be used to obtain sufficient sequence coverage, but the lengths of reads might be too short to allow de novo assembly, and the method of mapping to the reference HCV genome could be detecting the full-length HCV sequence. NGS technology is a powerful tool for obtaining more profound insight into the dynamics of genetic variants in the HCV quasispecies in human serum.[26]

Currently, potential treatments in development include drugs that target the HCV NS3/4A serine protease and the NS5B RNA-dependent RNA polymerase referred to as direct-acting antiviral agent (DAA).[27] These drugs have been evaluated in clinical trials alone and in combination with pegylated interferon and ribavirin.[28] Therefore, detecting the frequency of drug-resistant HCV variants prior to treatment is important. In treatment-naïve patients, the frequency of all resistant variants of NS3 was generally found to be below 1% using the 454 GS series[29,30] or by the Illumina Genome sequencer.[31] Viral dynamics have emerged whereby drug-resistant variants frequently appear, but are rapidly lost in the absence of selective pressure because of reduced fitness. Results using NGS technology have also suggested that not only the number but also the nature of the nucleotide changes can contribute to the genetic barriers to the development of resistance to DAAs.[32] Using a genetically engineered HCV infection system in a chimeric mouse model, the rapid emergence of DAA-resistant HCV variants was induced by mutation from a wild-type strain of HCV *in vivo*.[33]

Other 454 GS series sequencer studies showed that analysis of the PKR-elf2α phosphorylation homology domain sequence before or during treatment cannot be used to reliably predict the outcome of treatment in patients co-infected with HCV genotype 1 and HIV,[34] and highlighted the genetic diversity of HCV, which enables it to evade the host immune system.[35] Concerning the within-host evolution of HCV during infection, the genetic diversity of viral variants showed strong selective forces that limit viral evolution in the acute phase of infection.[36,37]

Hepatitis B virus (HBV)

Taking nucleoside/nucleotide analogs (NA) is a major antiviral therapy for the treatment of chronic HBV infection.[38] They inhibit the viral polymerase activity by interfering with the priming of reverse transcription and elongation of the viral minus or plus strand DNA.[39] The problem is that these treatments are hampered by the selection of drug-resistant mutants, leading to a loss of efficacy, viral relapse and exacerbations of hepatitis after discontinuation.[40]

Using NGS, drug-resistant mutations of HBV minor variants can be identified. The dynamics of emerging NA-resistant HBV variants are not well understood because standard Sanger sequencing methods detect drug-resistance mutations only after they have become dominant. NGS methods may offer significant advantages in explaining and predicting the responses of patients with HBV to antiviral therapy. In the sequential analysis of the region encoded reverse transcriptase, NA-resistant HBV variants were present in combinations of amino acid substitutions that increased in complexity after viral breakthrough or unsuccessful therapy with NA, at which time the combined NA-resistant variants predominated and the pretreatment HBV variants did not show NA-resistant motifs.[41–43] In another study, primarily NA resistance-related mutant variants were found to exist with minor variants in treatment-naïve patients.[44]

Hepatocellular carcinoma (HCC)

Despite its global importance, HCC is understudied compared to other major lethal cancers and we have a

little knowledge of the genomic alterations related to the initiation and progression of HCC. This may be due to the high complexity of the HCC cancer genome, which simple genomic approaches cannot easily simplify. Previous studies have revealed several genetic aberrations in HCC, including point mutations in p53[45] and Wnt-activating β-catenin,[46] hepatocyte-specific Pten deficiency,[47] the interaction of c-Myc and transforming growth factor (TGF)-α,[48] overexpression of the proto-oncogene MET[49] or cyclin D1/TGF-β1,[50] and HBV integrations into the TERT and MLL4 gene loci that encode telomerase reverse transcriptase and histone lysine methyl transferase, respectively. The gene expression profiles of HCC have been gradually revealed and suggest the therapeutic potential for genetic targets.[51] However, knowledge of the genetic background in HCC is far from complete and the molecular changes of HCC tumorigenesis remain poorly understood. We have summarized the HCC information concerning related genes discovered by NGS technology from Europe and Asia. In 2012, Fujimoto *et al.* detected that multiple chromatin regulators, including *ARID1A*, *ARID1B*, *ARID2*, *MLL* and *MLL3*, were mutated in approximately 50% of the HCC and the HBV genome was frequently integrated in the *TERT* locus, as determined by whole-genome sequencing analysis by Illumina NGS sequencers.[52] A European group also found new recurrent alternations of *ARID1A*, *RPS6KA3*, *NFE2L2* and *IRF2*. In addition, Wnt/β-catenin signaling, related to the mutations of *RPS6KA3-AXIN1* and *NFE2L2-CTNNB1*, may be involved in liver carcinogenesis together with both oxidative stress metabolism and Ras/mitogen-activated protein kinase (MAPK).[53]

CONCLUSION

GIVEN THE RAPID development of NGS systems, the goal of determining a whole-genome sequence for $US 1000 could become feasible in the near future. The cost of sequencing has become greatly reduced, and "one cell" or "one molecule" sequencing has become possible. By performing whole-genome sequencing, RNA sequencing and epigenetic analysis at one cellular level, dynamic genomic changes can be followed with time-course analysis in the same cells or for differences between various cells. When analyzing the genome sequence by removing the cancer tissue, the data of a mixture of cancer cells and normal cells can be usually obtained. Cancer cells are usually changed at the genomic level; therefore, mixture sequence data of multiple species can be obtained in some cases. If the genome sequence of the cancer tissue can be determined at a one-cell level, we will obtain a more accurate understanding of the progress and development of the cancer. Moreover, with the development of the NGS systems, analysis of DNA and RNA sequencing at the intracellular level proceed. Single molecule sequencing of cDNA converted to mRNA by the Nanopore sequencer can accurately represent the structure of the mixed mRNA containing splicing variants and clarify their intracellular distribution. Using short-read NGS, a large number of sequence reads are obtained making it possible to analyze variants or mutants in the virus population. The application of this novel technique includes the profiling of disease-specific gene expressions. Recently, we have successfully demonstrated that serum samples from patients with primary biliary cirrhosis had a distinct miRNA expression profile using NGS.[54] As such technologies develop further, new applications can also be expected to appear.

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Sofosbuvir plus ribavirin in Japanese patients with chronic genotype 2 HCV infection: an open-label, phase 3 trial

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SUMMARY. Genotype 2 hepatitis C virus (HCV) accounts for up to 30% of chronic HCV infections in Japan. The standard of care for patients with genotype 2 HCV – peginterferon and ribavirin for 24 weeks – is poorly tolerated, especially among older patients and those with advanced liver disease. We conducted a phase 3, open-label study to assess the efficacy and safety of an all-oral combination of the NS5B polymerase inhibitor sofosbuvir and ribavirin in patients with chronic genotype 2 HCV infection in Japan. We enrolled 90 treatment-naïve and 63 previously treated patients at 20 sites in Japan. All patients received sofosbuvir 400 mg plus ribavirin (weight-based dosing) for 12 weeks. The primary endpoint was sustained virologic response at 12 weeks after therapy (SVR12). Of the 153 patients enrolled and treated, 60% had HCV genotype 2a, 11% had cirrhosis, and 22% were over the aged 65 or older. Overall, 148 patients (97%) achieved SVR12. Of the 90 treatment-naïve patients, 88 (98%) achieved SVR12, and of the 63 previously treated patients, 60 (95%) achieved SVR12. The rate of SVR12 was 94% in patients with cirrhosis and in those aged 65 and older. No patients discontinued study treatment due to adverse events. The most common adverse events were nasopharyngitis, anaemia and headache. Twelve weeks of sofosbuvir and ribavirin resulted in high rates of SVR12 in treatment-naïve and previously treated patients with chronic genotype 2 HCV infection. The treatment was safe and well tolerated by patients, including the elderly and those with cirrhosis.

Keywords: Hepatitis C virus, HCV genotype 2, direct-acting antiviral agents, nucleotide polymerase inhibitor.

INTRODUCTION

Approximately two million people in Japan – nearly 2% of the population – are chronically infected with the hepatitis C virus (HCV) [1]. The population of patients with chronic HCV infection in Japan differs from that of other countries; patients are generally older, have more advanced liver disease and are more likely to have received previous treatment for HCV infection [2,3]. It is estimated that 15–30% of Japanese patients with HCV will develop serious complications, including liver cirrhosis, end-stage liver disease and hepatocellular carcinoma [4]. Although genotype 1 HCV is currently the most prevalent strain of the virus in Japan, genotype 2 HCV, which now accounts for up to 30% of infections, is rising in prevalence [5]. The current standard of care regimen for the treatment of chronic genotype 2 HCV infection in Japan is 24 weeks of pegylated interferon alpha (Peg-IFNα) and ribavirin (RBV) [6]. Although relatively high rates of SVR

Abbreviations: CI, confidence interval; GCP, Good Clinical Practice; HCV, hepatitis C virus; ICH, International Conference on Harmonization; Peg-IFNα, pegylated interferon alpha; PK, pharmacokinetics; RBV, ribavirin; SVR12, 12 weeks after therapy.

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have been reported in clinical trials with this regimen (71–86%), the use of Peg-IFNα+RBV in an ageing population with progressive liver disease is limited by safety and tolerability issues. Moreover, a substantial number of patients have absolute or relative contraindications to interferon. As a result, many Japanese patients with chronic genotype 2 HCV infection have no available treatment options and are thus at risk for worsening of liver disease and complications of cirrhosis, including hepatocellular carcinoma.

Sofosbuvir (Gilead Sciences) is an oral nucleotide analogue inhibitor of the HCV-specific NS5B polymerase that has recently been approved in the United States and Europe for the treatment of chronic HCV infection [7]. The labelled use for patients with chronic genotype 2 HCV infection is sofosbuvir and RBV for 12 weeks. In phase 3 studies, 12 weeks of treatment with sofosbuvir plus RBV in patients infected with genotype 2 HCV resulted in rates of SVR12 of 97% in treatment-naïve patients, 93% in patients ineligible to receive interferon and 86–90% in previously treated patients [8–10].

We conducted a phase 3 trial to determine the efficacy and safety of 12 weeks of sofosbuvir and RBV in treatment-naïve and previously treated Japanese patients with chronic genotype 2 HCV infection with and without compensated cirrhosis.

METHODS

Patients

Patients were enrolled between 16 July 2013 and 30 September 2013 at 20 sites in Japan. Eligible patients were aged 20 years or older with a body weight of at least 40 kg. Patients were required to be chronically infected with genotype 2 HCV and with HCV RNA levels $\geq 10^4$ IU/mL at screening. Planned enrolment was for approximately 84 treatment-naïve and 50 previously treated patients. See Supplement for definitions of types of response to prior treatment.

Up to 40% of enrolled subjects in each group (i.e. treatment naive or treatment experienced) could have evidence of compensated cirrhosis at screening (Child-Pugh A). Cirrhosis was defined as liver biopsy showing a Metavir score of 4 or Ishak score ≥5 or a FibroScan score of >12.5 kPa. Patients were required to have ALT and AST ≤10 × upper limit of the normal range, platelet count ≥50 000 per μL, haemoglobin ≥11 g/dL for women and ≥12 g/dL for men and albumin ≥3 g/dL. There were no upper limits on age or body mass index. Similarly, no restriction was applied to white blood cell or absolute neutrophil count at screening.

Study design

In this multicenter, open-label trial, all patients received 12 weeks of treatment with 400 mg of sofosbuvir, administered orally once daily, and ribavirin (Copegus®, Chugai Pharmaceutical Co., Ltd, Tokyo, Japan), administered orally twice daily, with doses determined according to body weight (600 mg daily in patients with a body weight of ≤60 kg, 800 mg daily in patients weighing >60 and ≤80 kg, and 1000 mg daily in patients with a body weight of >80 kg).

In addition to the main study of efficacy and safety, sparse PK samples were collected from all patients over the course of the study for population PK analyses and all patients were eligible to participate in an optional substudy to determine the steady-state pharmacokinetics (PK) of sofosbuvir (and its predominant circulating metabolite GS-331007). The target enrolment per treatment group was approximately 15 patients. For the PK substudy, intensive serial pharmacokinetic samples were collected (samples obtained over 24 h postdose) at either the week 2 or week 4 treatment visits.

Study assessments

Screening assessments included serum HCV RNA levels and IL28B (rs12979860) genotyping, as well as standard laboratory and clinical tests. Serum HCV RNA was measured with the COBAS® TaqMan® HCV Test, version 2.0 for Use with the High Pure System (Roche Molecular Systems, West Sussex, UK), which has a lower limit of quantification (LLOQ) of 25 IU/mL. HCV genotype and subtype were determined at screening using the Siemens VERSANT HCV Genotype INNO-LiPA 2.0 assay.

On-treatment assessments included standard laboratory testing, serum HCV RNA, vital signs, electrocardiography and symptom-directed physical examinations. All adverse events were recorded and graded according to a standardized scale (see Supplementary Table S7).

NS5B amplification and deep sequencing was performed at DDL Diagnostics Laboratory (Rijswijk, The Netherlands) for all subjects who did not achieve SVR12. Deep sequencing of HCV NS5B was performed at the first virologic failure time point if a plasma/serum sample was available and HCV RNA was >1000 IU/mL, along with the respective baseline samples. Amino acid substitutions in NS5B in the samples collected at virologic failure were compared with the genotype 2 reference and the respective baseline sequence for each patient.

The population pharmacokinetic parameters for sofosbuvir and GS-331007 were computed for all subjects from concentration data from intensive and/or sparse samples using the previously established sofosbuvir and GS-331007 population PK models [11].

Statistical analysis

For treatment-naïve patients without cirrhosis, the SVR12 rate was compared to an adjusted historical SVR rate of 69%, using a two-sided exact one-sample binomial test. The historical control rate was calculated from the weighted average of historical SVR rates for noncirrhotic,

treatment-naïve Japanese patients with genotype 2 HCV infection receiving 24 weeks of Peg-IFNα+RBV (79% with a 10% discount applied due to the expected improvement in safety profile and shorter treatment duration – see Supplementary Table S2 for further details). We calculated that a sample size of 50 patients would provide 80% power to detect an 18% improvement in the SVR12 rate over the adjusted historical rate at a significance level of 0.05. For SVR12 rates for the overall population, for treatment-naïve patients with cirrhosis, and for previously treated patients, statistical hypothesis testing was not performed. For these outcomes, we calculated point estimates of SVR12 rates with two-sided 95% exact confidence interval using the binomial distribution (Clopper–Pearson method).

Study oversight

This trial was approved by the institutional review board or independent ethics committees at all participating sites and was conducted in accordance with local regulations and with recognized international scientific and ethical standards, including the International Conference on Harmonization (ICH) guideline for Good Clinical Practice (GCP) and the original principles embodied in the Declaration of Helsinki. The study was designed and conducted according to protocol by the sponsor (Gilead Sciences) in collaboration with the principal investigators. The sponsor collected the data, monitored study conduct and performed the statistical analyses. The manuscript was prepared by Gilead Sciences with input from all authors.

RESULTS

Baseline characteristics

Of the 188 patients who were initially screened, 153 (90 treatment-naïve and 63 previously treated patients) were enrolled and began treatment (Table S1 and Figure S1). The demographic and baseline clinical characteristics of the patients are provided in Table 1. Overall, the majority of patients were female (54%), and all were Japanese. The mean age was 57 years (ranging from 25 to 74 years) and 22% were aged 65 or older.

Previously treated patients were slightly older than the treatment-naïve patients, with a higher percentage of males, higher baseline viral load, with a higher prevalence of cirrho-

Table 1 Baseline Demographic Characteristics

Characteristic	Overall (N = 153)	Treatment naïve (n = 90)	Previously treated (n = 63)
Mean age, years (range)	57 (25, 74)	55 (25, 73)	60 (34, 74)
Mean BMI, kg/m^2 (range)	24 (16.5, 34)	24 (17, 34)	24 (16.5, 34)
Male, n (%)	70 (46)	33 (37)	37 (59)
Mean HCV RNA, $\log_{10}$ IU/mL ± SD	6.3 (0.84)	6.2 (0.92)	6.5 (0.66)
HCV RNA ≥5 $\log_{10}$ IU/mL, n (%)	140 (92)	78 (87)	62 (98)
HCV genotype, n (%)			
2a	92 (60)	52 (58%)	40 (63%)
2b	61 (40)	38 (42%)	23 (37%)
Cirrhosis, n (%)			
No	136 (89)	82 (91)	54 (86)
Yes	17 (11)	8 (9)	9 (14)
IL28B genotype, n (%)			
CC	121 (79)	73 (81)	48 (76)
CT	28 (18)	17 (19)	11 (17)
TT	4 (3)	0	4 (6)
Median baseline ALT, U/L (range)	34 (12, 412)	32 (12, 412)	36 (12, 232)
Baseline ALT >1.5 × ULN, n (%)	43 (28)	28 (31)	15 (24)
Interferon eligibility, n (%)*			
Interferon eligible	72 (80)	72 (80)	Not applicable
Interferon ineligible	5 (6)	5 (6)	Not applicable
Interferon unwilling	13 (14)	13 (14)	Not applicable
Response to prior HCV treatment, n (%)			
Nonresponse	15 (24)	Not applicable	15 (24)
Relapse/breakthrough	45 (71)	Not applicable	45 (71)
Interferon intolerant	3 (5)	Not applicable	3 (5)
Median eGFR, mL/min (range)	85 (51, 209)	86 (52, 175)	84 (51, 209)

*Interferon eligibility was determined by the site investigator based on whether or not, in their judgment, the patient had contraindications to interferon therapy.

sis and non-CC IL28B genotype. Overall, 11% of participating subjects had cirrhosis. The proportions of patients infected with genotype 2a and 2b HCV were 60% and 40%, respectively, which is similar to previous reports of HCV subtype distribution in the Japanese population [4]. Most (80%) of the treatment-naïve patients were considered eligible for interferon therapy, with 6% having contraindications to interferon therapy and 14% unwilling to receive this treatment. Most (71%) of the previously treated patients had experienced virologic breakthrough or relapse after previous treatment, with 24% reporting nonresponse to prior therapy.

Efficacy

Overall, 148 of the 153 patients (97%, 95% confidence interval [CI] 93–99%) achieved SVR12 (Table 2). By prior treatment history, 88 of the 90 treatment-naïve patients (98%, 95% CI, 92–100%) and 60 of the 63 previously treated patients (95%, 95% CI, 87–99%) achieved SVR12. Of the 82 treatment-naïve patients without cirrhosis, 80 (97%, 95% CI 91–100%) achieved SVR12, thus meeting the primary efficacy endpoint for this group of superiority to the adjusted historical control rate of 69% ($P < 0.001$). Of note, all eight treatment-naïve patients (100%) with cirrhosis and eight of the nine previously treated patients with cirrhosis (89%) achieved SVR12. Overall, 16 of the 17 patients with cirrhosis (94%, 95% CI 71–100%) achieved SVR12.

Patient responses according to baseline characteristics are shown in Supplementary Table S3. Rates of SVR12 were high in all subgroups of patients. Patients with characteristics historically associated with poor response to interferon-based treatment – non-CC IL28B genotype, high baseline viral load, elderly patients, cirrhosis – had rates of SVR12 similar to those in patients without these characteristics.

Relapse accounted for all cases of virologic failure; there were no patients with virologic breakthrough or nonresponse during treatment. Among all patients treated, 97% had HCV RNA <LLOQ by treatment week 2, and 100% achieved HCV RNA <LLOQ by treatment week 4. Overall, five patients experienced virologic relapse after the end of therapy: two (2%) treatment-naïve patients and three (5%) treatment-experienced patients. Four patients relapsed by post-treatment week 4, and one patient relapsed between post-treatment weeks 4 and 12. Characteristics of patients who relapsed are provided in Table S4. There were no consistent host or viral characteristics in the five subjects who relapsed; however, the number of virologic failures is too small for any conclusions to be drawn concerning predictors of virologic failure. No patient relapsed after post-treatment week 12. All 148 SVR12 patients (100%) also achieved SVR24.

Viral resistance testing

The NS5B region was deep sequenced in samples collected from the five relapsers at baseline and at the time of relapse. No S282T variant – known to be associated with reduced susceptibility to sofosbuvir – or any other nucleotide inhibitor resistance-associated variants were detected in any patient at relapse. Phenotypic analysis of the NS5B gene showed no change in susceptibility to either sofosbuvir or ribavirin.

Pharmacokinetics

Population pharmacokinetic analysis was performed to estimate the pharmacokinetics of sofosbuvir and its major circulating nucleoside metabolite, GS-331007. The mean (CV%) of steady-state AUC_{0-24} and C_{max} were 973 (31.2) ng*h/mL and 544 (33.6) ng/mL for sofosbuvir ($N = 45$), respectively, and 10 400 (27.2) ng h/mL and 818 (27.9) ng/mL for GS-331007 ($N = 153$), respectively. Within the Japanese study population, there were no clinically relevant differences in the pharmacokinetics of GS-331007 and sofosbuvir, based on age, sex, BMI, cirrhosis status, prior treatment experience or SVR12 outcome.

Safety

Overall, 73% of patients experienced at least one adverse event; however, the majority of patients experiencing

Table 2 Response during and after Treatment

Response	Overall ($N = 153$)	Treatment naïve ($n = 90$)	Previously treated ($n = 63$)
HCV RNA <LLOQ during treatment, *n* (%)*			
At week 2	148 (97%)	88 (98%)	60 (95%)
At week 4	153 (100%)	90 (100%)	63 (100%)
HCV RNA <LLOQ after end of treatment, *n* (%)			
SVR4	149 (97%)	89 (99%)	60 (95%)
SVR12	148 (97%)	88 (98%)	60 (95%)
95% confidence interval	92.5–99%	92–>99%	87–99%
On-treatment failure	0	0	0
Relapse, *n/n* (%)	5 (3%)	2 (2%)	3 (5%)

*LLOQ denotes lower limit of quantification, which is 25 IU/mL. SVR denotes sustained virologic response.

adverse events (84%) had only mild (grade 1) events. The most common treatment-emergent adverse events were nasopharyngitis (upper respiratory viral illness), anaemia, headache, malaise and pruritus (Table 3). No patient in the study discontinued treatment prematurely due to adverse events (or for any other reason). Twenty-two patients (14%) had adverse events that led to modification or interruption of a study drug: 20 patients had ribavirin dose reductions to manage anaemia, and one patient interrupted sofosbuvir and RBV for 1 day because of an event of nasopharyngitis. All but one of the 22 patients with modification or interruption of study drugs achieved SVR12. Two patients experienced treatment-emergent serious adverse events: one treatment-experienced 63-year-old woman had a worsening of anaemia for which she was hospitalized, and one treatment-naïve 36-year-old woman had a severe anaphylactic reaction to a bee sting. No patient experienced a life-threatening (grade 4) adverse event, and only three patients experienced severe (grade 3) events, two of which were deemed to be related to study treatment, the above-mentioned case of anaemia and one case of transient, ribavirin-associated hyperbilirubinaemia in a treatment-experienced 65-year-old man, which resolved during follow-up.

The overall rates of adverse events in younger (<65 years) and older (≥65 years) patients did not differ substantially (72% *vs* 76%, respectively), although there was a higher incidence of anaemia and pruritus in older patients (Table S5). The incidence and severity of adverse events in patients with and without cirrhosis at baseline were similar (Table S6).

Table 3 Discontinuations, Adverse Events and Laboratory Abnormalities by Age

Parameter	Overall (N = 153)
Discontinuation of any study drug due to adverse event	0
Serious adverse events	2 (1%)
Anaemia	1 (1%)
Anaphylactic reaction	1 (1%)
Any adverse event	112 (73%)
Common adverse events*	
Nasopharyngitis	45 (29%)
Anaemia	18 (12%)
Headache	15 (10%)
Malaise	11 (7%)
Pruritus	9 (6%)
Laboratory abnormalities, n (%)	
Decreased haemoglobin concentration	
<10 g/dL	19 (12%)
<8 g/dL	1 (1%)
Neutropenia (500–<750 per mm^3)	1 (1%)
Hyperglycaemia (>250–500 mg/dL)	3 (2%)
Hyperbilirubinaemia (>2.5–5.0 × ULN)	2 (1%)

ULN, upper limit of normal.
*Adverse events occurring in at least 5% of patients.

Overall, the mean change in haemoglobin from baseline to week 12 of treatment was −1.2 g/dL. For patients aged 65 and older, the mean change in haemoglobin was −1.7 g/dL, as compared with 1.0 g/dL in patients under the age of 65. Of all 153 patients enrolled and treated, 19 (12%) had at least one postbaseline haemoglobin value of <10.0 g/dL, and one (1%) had a postbaseline haemoglobin value of <8.5 g/dL. Two patients (1%) had grade 3 hyperbilirubinaemia; no grade 4 hyperbilirubinaemia occurred. One patient, who had grade 2 neutropenia at baseline, had transitory grade 3 neutropenia.

DISCUSSION

In this phase 3 trial, twelve weeks of treatment with sofosbuvir and RBV resulted in high rates of sustained virologic response (>95%) in treatment-naïve and previously treated Japanese patients with chronic genotype 2 HCV infection. Patients with host and viral characteristics that have historically been predictive of lower rates of SVR – older age, presence of cirrhosis, high viral load, non-CC IL28B alleles – had rates of SVR12 similar to patients without these characteristics. In patients who had been previously treated for HCV infection, the nature of the prior response was not associated with significant differences in rates of SVR following treatment with sofosbuvir and ribavirin; patients who had nonresponse to prior treatment had similar response rates as patients who had previously experienced relapse or viral breakthrough. No clear or consistent baseline predictors of treatment failure were evident among the five patients who relapsed after treatment.

The current standard-of-care treatment for Japanese patients with chronic genotype 2 HCV infection is 24 weeks of Peg-IFNα+RBV. Although patients who received this regimen in clinical trials achieved SVR12 rates ranging from 72% to 86%, these studies were restricted to patients <65 years of age [12,13]. However, the Japanese population chronically infected with genotype 2 HCV includes many patients with characteristics that make the use of interferon-based therapy problematic – older age, progressive liver disease, prior treatment experience and comorbid conditions such as diabetes and cardiovascular disease [14]. Moreover, many patients cannot receive interferon therapy due to relative or absolute contraindications. The interferon-free combination of sofosbuvir and ribavirin may represent a promising treatment option for these patients.

Given the characteristics of the patient population in Japan with HCV infection – generally older, and more likely to have advanced liver disease – safety and tolerability of therapeutic regimens is an important issue. In the present study, 22% of patients were aged 65 or older and 11% had cirrhosis. Analyses of safety data by age (<65 *vs*

≥65 years) showed increases in reported adverse events and laboratory abnormalities in older patients, but these differences did not present a barrier to treatment as no premature discontinuation of study treatment occurred in any patient. Analysis of safety data according to the presence or absence of cirrhosis did not indicate clinically important differences in safety or tolerability of the 12-week sofosbuvir plus ribavirin regimen.

Consistent with previous reports, the results of this study confirm the high barrier to resistance afforded by the sofosbuvir plus RBV treatment regimen. Rapid viral suppression was observed with all patients achieving HCV RNA undetectable status by week 4, with no virologic breakthrough observed during treatment in any of the 153 patients. The percentage of patients who relapsed after treatment was low (3%), and none of the subjects who relapsed had S282T or other nucleoside inhibitor resistance-associated variants. No change in susceptibility to sofosbuvir or ribavirin compared with the corresponding baseline or wild-type reference was observed at the relapse time point.

The main limitation of this study was the lack of a control arm to allow direct comparison with interferon-based regimens. Several considerations guided our choice of an uncontrolled study design. Adding an interferon-based control arm would have required exclusion of patients who were ineligible to receive or intolerant of interferon – an important and substantial proportion of patients – as well as previously treated patients, for whom further interferon treatment is not an option. Moreover, given that Peg-IFNα is administered by subcutaneous injection, blinding of treatment arms would not have been possible.

In conclusion, treatment with the all-oral, interferon-free combination of sofosbuvir and RBV resulted in high rates of sustained virologic response in both treatment-naïve and previously treated Japanese patients with chronic genotype 2 HCV infection. The degree of antiviral efficacy coupled with a favourable safety and tolerability profile, including patients with cirrhosis and those aged 65 and older, suggest that this combination may fill an important unmet medical need in Japan.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Patient disposition.

Table S1. Reasons for screen failure.

Table S2. Calculation of the adjusted historical control rate.

Table S3. SVR12 by subgroup.

Table S4. Characteristics of patients who relapsed.

Table S5. Common adverse events by age group.

Table S6. Common adverse events by cirrhosis status.

Table S7. Gilead sciences grading scale for severity of adverse events and laboratory abnormalities.

Hepatology Research

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Original Article

Interleukin 28B polymorphism predicts interferon plus ribavirin treatment outcome in patients with hepatitis C virus-related liver cirrhosis: A multicenter retrospective study in Japan

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Aim: This study evaluated the efficacy of interferon plus ribavirin and examined whether interleukin 28B (IL28B) polymorphism influenced treatment outcome in Japanese patients with hepatitis C virus (HCV)-related liver cirrhosis (LC).

Methods: Fourteen collaborating centers provided details of 261 patients with HCV-related LC undergoing treatment with interferon plus ribavirin. Univariate and multivariate analyses were used to establish which factors predicted treatment outcome.

Results: Eighty-four patients (32.2%) achieved a sustained virological response (SVR). SVR rates were 21.6% (41/190) in patients with HCV genotype 1 with high viral load (G1H) and 60.6% (43/71) in patients with non-G1H. In patients with non-G1H, treatment outcome was effective irrespective of IL28B polymorphism. In those with G1H, SVR was achieved in 27.1% of patients with the IL28B rs8099917 TT allele compared with 8.8% of those with the TG/GG alleles ($P = 0.004$). In patients with G1H having TT allele, treatments longer than 48 weeks achieved significantly higher SVR rates than treatments less than 48 weeks (34.6% vs 16.4%, $P = 0.042$). In patients with G1H having TG/GG alleles, treatments longer than 72 weeks achieved significantly higher SVR rates than treatments less than 72 weeks (37.5% vs 4.1%, $P = 0.010$).

Conclusion: Interferon plus ribavirin treatment in Japanese patients with non-G1H HCV-related LC was more effective than those with G1H and not influenced by IL28B polymorphism. In those with G1H, IL28B polymorphism may predict SVR and guide treatment duration: SVR rates were higher in those with the TT allele treated for more than 48 weeks and those with the TG/GG alleles treated for more than 72 weeks.

Key words: cirrhosis, hepatitis C virus, interferon, interleukin 28B, ribavirin

INTRODUCTION

CHRONIC HEPATITIS C virus (HCV) infection is a leading cause of liver cirrhosis worldwide.[1] Patients with HCV-related liver cirrhosis (LC) are at increased risk of hepatic decompensation and hepatocellular

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carcinoma (HCC).[2–4] The therapeutic goal in these patients should be the prevention of liver-related mortality. A randomized trial conducted in Japan was the first to suggest that interferon (IFN) may reduce the risk of HCC in patients with HCV-related LC.[5] Recent studies have shown that patients with HCV-related LC who achieved a sustained virological response (SVR) with antiviral therapy had a significant reduction in liver-related mortality.[6,7] However, patients with HCV-related LC show a lower SVR rate than non-cirrhotic patients, as well as a reduced tolerance to the therapy.[8,9] A previous meta-analysis revealed that the overall SVR rate in patients with cirrhosis was 33.3%, and was significantly higher in patients with HCV genotypes 2 and 3 (55.4%) than in those with HCV genotypes 1 and 4 (21.7%).[10]

Genome-wide association studies have recently shown that single nucleotide polymorphisms (SNP) near the interleukin 28B (IL28B) region (rs8099917, rs12979860) are the most powerful predictors of SVR to pegylated (PEG) IFN plus ribavirin in patients with HCV genotype 1 infection.[11–13] However, it is not clear whether IL28B polymorphism can be used to predict the virological response to treatment of HCV-related LC. This study evaluated the efficacy of IFN plus ribavirin, and the association between IL28B polymorphism and the treatment efficacy in Japanese patients with HCV-related LC.

METHODS

THIS WAS A multicenter retrospective study of patients with HCV-related LC who had received treatment with IFN plus ribavirin in 14 hospitals in Japan.

Patient selection

Data were collected from 290 patients with HCV-related LC receiving treatment with IFN plus ribavirin in 14 academic and community hospitals. All patients had compensated HCV-related LC with clinical or histological data available. The diagnosis of cirrhosis met at least one of the following criteria: liver biopsy specimens with cirrhosis, diffuse formation of the nodules on the liver surface in peritoneoscopy, over 12.5 kPa in liver stiffness values on transient elastography, signs of portal hypertension on ultrasound scan (splenomegaly, portal vein enlargement, re-permeabilization of the umbilical vein, or presence of portal-systemic shunts), presence of esophageal varices on endoscopy or positive values using the following discriminant by Ikeda and colleagues: $z = 0.124 \times (\gamma\text{-globulin [\%]}) + 0.001 \times$ (hyaluronate) ($\mu g\ L^{-1}$) $- 0.075 \times$ (platelet count [$\times 10^4$ counts/mm^3]) $- 0.413 \times$ sex (male, 1; female, 2) $- 2.005$.[14–16] Principal investigators in 14 hospitals identified eligible patients and entered data in a predefined database.

Combination therapy

Of the 290 patients identified, 29 were not genotyped for IL28B SNP, thus the data of 261 patients were analyzed. A total of 190 patients were infected with HCV genotype 1 with high viral load (>100 KIU/mL) (G1H) (72.8%) and the remaining 71 (27.2%) were classified as non-G1H. Twenty-two patients were HCV genotype 1 with low viral load, 46 were genotype 2a or 2b, and three were of unknown genotype. Two hundred and twenty-four (85.8%) patients were treated with PEG IFN-α-2b (1.5–1.0 μg/kg bodyweight per week), 20 (7.7%) patients were treated with PEG IFN-α-2a (45–180 μg/week) and the remaining 17 (6.5%) patients were treated with IFN-α-2b or IFN-β. IFN-α-2b and IFN-β were administrated at a median dose of 6 million units each day (seven times per week for the initial 2 or 4 weeks, followed by three times per week thereafter). All patients also received oral ribavirin (600–1000 mg/day). Median treatment duration was 48 and 28 weeks in G1H and non-G1H, respectively. The individual attending physician determined the treatment regimes and their duration.

Virological response during therapy and definitions

The efficacy end-point was SVR, defined as undetectable serum HCV RNA 24 weeks after treatment. Relapse was defined as undetectable serum HCV RNA at the last treatment visit but detectable serum HCV RNA again at the last follow-up visit. Breakthrough was defined as reappearance of serum HCV RNA during treatment. A non-responder was defined as serum HCV RNA never undetectable during treatment. A rapid virological response (RVR) was defined as undetectable serum HCV RNA at treatment week 4, and a complete early virological response (cEVR) was defined as undetectable serum HCV RNA at treatment week 12. A late virological response (LVR) was defined as detectable serum HCV RNA at 12 weeks that became undetectable within 36 weeks of the start of treatment.

Determination of IL28B genotype

Interleukin 28B (rs8099917) was genotyped in each of the 14 hospitals by Invader assay, TaqMan assay or by direct sequencing, as previously described.[17,18]

Statistical analysis

Results were analyzed on the intention-to-treat principle. Mean differences were tested using Student's *t*-test. The difference in the frequency distribution was analyzed with Fisher's exact test. Univariate and multivariate logistic regression analyses were used to identify factors independently associated with SVR. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. The parameters that achieved statistical significance on univariate analysis were entered into multivariate logistic regression analysis to identify significant independent factors. Data were analyzed with JMP version 9.0 for Macintosh (SAS Institute, Cary, NC, USA). All statistical analyses were two sided, and $P < 0.05$ was considered significant.

RESULTS

OF THE 261 patients included in our analysis, 84 patients (32.2%) achieved SVR (Fig. 1). The rate of relapse and breakthrough was 24.9% and the non-responder rate was 33.3%. There were 25 patients (9.6%) who required early discontinuation of treatment because of adverse events. Baseline demographic and clinical features are summarized in Table 1. The age of the patients was 60.7 ± 8.9 years and 50.6% were male. Of the patients studied, 125 patients (47.9%) had been treated with IFN previously, and 75 (28.7%) had not responded to previous treatment. One hundred and six patients (40.6%) had been treated for HCC before. There were 85 patients with esophageal varices (32.6%). There were 190 patients with G1H and 133 (70%) of these had the TT allele at IL28B rs8099917. There were 71 patients in the non-G1H group, 51 (71.8%) of whom were found to have the TT allele at IL28B rs8099917.

Virological response rates in patients with G1H and non-G1H HCV-related LC

The SVR rates were 21.6% (41/190) in patients with G1H and 60.6% (43/71) in patients with non-G1H (Table 2). There were no statistically significant differences between the G1H and non-G1H groups with regard to dose reduction rates of IFN or ribavirin. Dose reduction of IFN was required in 51.3% of patients and dose reduction of ribavirin in 53.6% of patients. Treatment duration in patients in the G1H group was significantly longer than those in the non-G1H group ($P = 0.010$).

Association between IL28B rs8099917 genotype and treatment response

Sustained virological response was achieved in 37.0% of patients with the rs8099917 TT allele and 20.8% in those with the TG or GG allele. Virological responses, including SVR, relapse and breakthrough, in patients with the rs8099917 TT allele were significantly higher than in those with rs8099917 TG or GG allele ($P = 0.013$ and 0.012, respectively; Table 3). The proportion of non-responders among patients with the rs8099917 TG or GG allele was significantly higher than in those with the TT allele ($P = 0.002$). There was no

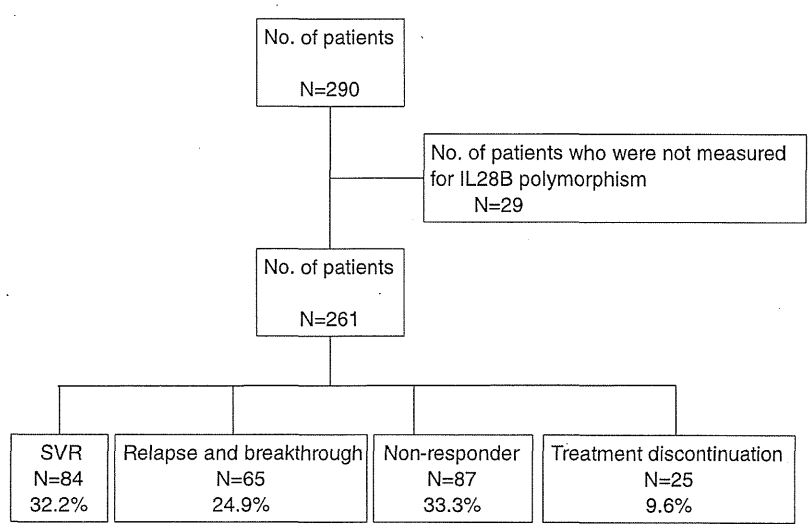

Figure 1 Flowchart showing the characteristics of the study cohort. IL28B, interleukin 28B; SVR, sustained virological response.

Table 1 Summary of demographic and baseline characteristics ($n = 261$)

	G1H, $n = 190$	Other than G1H, $n = 71$	All patients, $n = 261$
Sex (M : F)	95:95	37:34	132:129
Age (years)	60.5 ± 9.3	61.2 ± 7.8	60.7 ± 8.9
BMI (kg/m^2)	23.8 ± 3.5	23.4 ± 3.2	23.7 ± 3.4
IFN treatment history	91 (47.9%)	34 (47.9%)	125 (47.9%)
HCC treatment history	75 (39.5%)	31 (43.7%)	106 (40.6%)
Presence of EV	60 (31.6%)	25 (35.2%)	85 (32.6%)
Total bilirubin (mg/dl)	1.1 ± 0.9	1.1 ± 1.4	1.1 ± 1.2
AST (IU/L)	79.1 ± 44.2	75.8 ± 57.7	79.9 ± 52.7
ALT (IU/L)	82.4 ± 56.4	81.9 ± 75.4	83.3 ± 66.2
GGT (IU/L)	83.8 ± 107.8	87.0 ± 140.1	84.6 ± 115.8
Albumin (g/dL)	3.7 ± 0.5	3.8 ± 0.4	3.7 ± 0.5
Prothrombin (%)	86.2 ± 14.4	83.7 ± 16.7	85.5 ± 15.1
WBC (/μL)	4407 ± 1592	4190 ± 1930	4348 ± 1667
Hemoglobin (g/dL)	13.2 ± 1.8	13.1 ± 1.8	13.1 ± 1.8
Platelets (10^4/mm^3)	11.8 ± 6.7	11.8 ± 6.3	11.8 ± 6.6
AFP (ng/mL)	48.9 ± 224.7	24.0 ± 29.3	45.4 ± 193.9
DCP (mAU/mL)	66.8 ± 372.3	155.3 ± 620.4	92.4 ± 450.8
IL28B (TT : TG + GG)	133:57	51:20	184:77

All values are expressed as mean ± standard deviation.
AFP, α-fetoprotein; ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; DCP, des-γ-carboxy prothrombin; EV, esophageal varices; G1H, genotype 1 with high viral load; GGT, γ-glutamyltransferase; HCC, hepatocellular carcinoma; IFN, interferon; IL28B, interleukin 28B rs8099917 genotype; WBC, white blood cell.

significant association between the IL28B genotype and the incidence of adverse events.

Among patients in the G1H group, SVR was achieved in 27.1% (36/133) of those with the TT allele and 8.8% (5/57) of those with the TG or GG allele (Table 4). There was no statistically significant difference between IL28B genotype and viral response in patients with non-G1H.

Table 2 Summary of treatment and sustained virological response rates ($n = 261$)

	G1H, $n = 190$	Other than G1H, $n = 71$	All patients, $n = 261$
Dose reduction of IFN	$n = 98$ (51.6%)	$n = 36$ (50.7%)	$n = 134$ (51.3%)
Dose reduction of RBV	$n = 107$ (56.3%)	$n = 33$ (46.5%)	$n = 140$ (53.6%)
Treatment duration (weeks)			
Mean ± SD	45.3 ± 21.6	37.7 ± 19.6	43.2 ± 21.4
Median	48	28	48
SVR	$n = 41$ (21.6%)	$n = 43$ (60.6%)	$n = 84$ (32.2%)

G1H, genotype 1 with high viral load; IFN, interferon; RBV, ribavirin; SD, standard deviation; SVR, sustained virological response.

Table 3 Association between IL28B rs8099917 polymorphism and treatment response in 261 hepatitis C virus-related liver cirrhotic patients

IL28B	TT ($n = 184$)	TG + GG ($n = 77$)	*P*-value
SVR	68 (37.0%)	16 (20.8%)	0.013
Relapse and breakthrough	54 (29.3%)	11 (14.3%)	0.012
Non-responder	44 (23.9%)	43 (55.8%)	0.002
Discontinuation	18 (9.8%)	7 (9.1%)	1.000

IL28B, interleukin 28B rs8099917 genotype; SVR, sustained virological response.

Table 4 Sustained virological response associated between IL28B rs8099917 polymorphism and G1H in hepatitis C virus-related liver cirrhosis patients

IL28B	TT ($n = 184$)	TG + GG ($n = 77$)	*P*-value
G1H	36/133 (27.1%)	5/57 (8.8%)	0.004
Other than G1H	32/51 (62.7%)	11/20 (55.0%)	0.596

G1H, genotype 1 with high viral load; IL28B, interleukin 28B rs8099917 polymorphism.

Predictive factors associated with SVR

Differences in the characteristics of patients with SVR and those in whom SVR was not achieved are summarized in Table 5. Neither age, sex, alanine transaminase, aspartate aminotransferase, prothrombin activity, hemoglobin nor platelet counts appeared to significantly influence the chance of achieving SVR. The patients who achieved SVR had a lower body mass index, higher white blood cell count and higher serum albumin than those who did not, and were more likely to have non-G1H and the TT allele of IL28B rs8099917. Multivariate analysis identified that possession of the IL28B rs8099917 TT allele (OR = 2.85; 95% CI, 1.01–9.15; $P = 0.047$) and non-G1H (OR = 6.49; 95% CI, 1.77–26.43; $P = 0.005$) as significant determinants of SVR.

Treatment duration and efficacy in patients with G1H

Of the patients with G1H, 79 (41.6%) received less than 48 weeks of treatment. The number receiving 48–52 weeks, 53–72 weeks, over 72 weeks and unknown duration of treatment were 54 (28.4%), 41 (21.6%), 14 (7.4%) and two (1.1%), respectively. The median duration of treatment in patients who achieved RVR and cEVR was 48 weeks, but was significantly longer (66 weeks) in those with an LVR ($P < 0.001$). Table 6 shows the SVR rates of those with different IL28B genotypes and on-treatment viral response. The SVR rate in patients who achieved LVR was significantly lower than those who achieved RVR and cEVR ($P = 0.002$). Of the patients with G1H found to have the IL28B TG or GG genotype, none achieved RVR and only two achieved cEVR.

Predictors of SVR in patients with G1H and the TT allele

Patients with G1H and the TT allele who achieved SVR had higher platelet counts, higher serum albumin and had undergone over 48 weeks of treatment. Multivariate analysis identified platelet count (OR = 1.08; 95% CI, 1.01–1.18; $P = 0.047$), serum albumin (OR = 2.78; 95% CI, 1.14–7.42; $P = 0.031$) and over 48 weeks of treatment duration (OR = 2.53; 95% CI, 1.07–6.49; $P = 0.042$) as significant determinants of SVR (Table 7).

Predictors of SVR in patients with G1H and the TG or GG allele

Patients who had G1H and the TG or GG allele who achieved SVR had a higher total dose of ribavirin ($P = 0.011$) and more than 72 weeks of treatment duration ($P = 0.010$).

Treatment tolerability and adverse events

Table 8 illustrates details of the patients who experienced adverse events higher than grade 2. There were

Table 5 Factors associated with sustained virological response in hepatitis C virus-related liver cirrhosis patients

Factors	SVR (+), ($n = 84$)	SVR (–), ($n = 177$)	*P*-value	Multivariate analyses		
				Odds ratio	95% CI	*P*-value
BMI (kg/m^2)	22.9 ± 3.5	24.0 ± 3.3	0.019			
WBC (/μL)	4727 ± 2096	4168 ± 1376	0.013			
Albumin (g/dL)	3.83 ± 0.48	3.68 ± 0.46	0.018			
Other than G1H	$n = 43$ (51.2%)	$n = 28$ (15.8%)	<0.001	6.49	1.77–26.43	0.005
IL28B TT	$n = 68$ (81.0%)	$n = 116$ (65.5%)	0.012	2.85	1.01–9.15	0.047

P-values were obtained by logistic regression model.
BMI, body mass index; CI, confidence interval; G1H, genotype 1 with high viral load; IL28B, interleukin 28B rs8099917 polymorphism; SVR, sustained virological response; WBC, white blood cell.

Table 6 Sustained viral response rates between IL28B genotype and on-treatment viral response in the patients with G1H

	IL28B TT	IL28B TG/GG	All patients
RVR	7/7	0/0	7/7
	100%	0%	100%
cEVR	15/26	1/2	16/28
	57.7%	50%	57.1%
LVR	14/44	4/11	18/55
	31.8%	36.4%	32.7%

cEVR, complete early virological response (defined as serum HCV RNA negative at treatment week 12); G1H, genotype 1 with high viral load; HCV, hepatitis C virus; IL28B, interleukin 28B rs8099917; LVR, late virological response (defined as serum HCV RNA detectable at 12 weeks and undetectable at 36 weeks after the start of treatment); RVR, rapid virological response (defined as serum HCV RNA negative at treatment week 4).

two cases of liver decompensation, two cases of interstitial pneumonia, one case of cerebral hemorrhage and one case of cerebral infarction. The cause of death in two patients was decompensation of LC. In one patient, treatment was stopped after 4 weeks, and in another, treatment was stopped after 32 weeks because of hepatic failure. The IFN dose was reduced in 134 patients (51.3%), and the ribavirin dose was reduced in 140 patients (53.6%) and discontinued in 60 patients (23.0%). Among patients who had treatment discontinued, 27 patients (10.3%) had treatment withdrawn because of no virological response and 33 patients (12.6%) because of severe adverse events. In patients in whom treatment was discontinued, three patients had SVR and five had a relapse.

IL28B alleles predicting SVR in G1H group

The influence of IL28B rs8099917 genotype on SVR in G1H is shown in Figure 2. Overall, there were 84 patients (32.2%) who achieved SVR with IFN plus ribavirin in HCV-related LC. The SVR was 60.6% in those with non-G1H, and was not significantly influenced by IL28B rs8099917 genotype (the SVR in TT patients was 62.7% compared with 55.0% in TG or GG patients). In contrast, in patients with G1H, the SVR of patients with IL28B rs8099917 genotype TT was significantly higher than those with rs8099917 TG or GG (27.1% vs 8.8%, $P = 0.004$). In patients with G1H and IL28B TT, the SVR of those treated for over 48 weeks was significantly higher than those treated for less than 48 weeks (34.6% vs 16.4%, $P = 0.042$). In patients with G1H and IL28B TG/GG, the SVR of those treated for over 72 weeks was significantly higher than those treated for less than 72 weeks (37.5% vs 4.1%, $P = 0.010$).

Table 8 Adverse events higher than grade 2

	No. of patients (%)
Anemia	63 (24.1%)
Thrombocytopenia	31 (11.9%)
Leukopenia	19 (7.3%)
Rash and itching	17 (6.5%)
Fatigue and general malaise	15 (5.7%)
Gastrointestinal disorders	5 (1.9%)
Depression	5 (1.9%)
Development of hepatocellular carcinoma	3 (1.1%)
Respiratory disorders	3 (1.1%)
Liver decompensation	2 (0.8%)
Malignant neoplasm	2 (0.8%)
Interstitial pneumonia	2 (0.8%)
Cerebral hemorrhage	1 (0.4%)
Cerebral infarction	1 (0.4%)
Cholangitis	1 (0.4%)
Retinal hemorrhage	1 (0.4%)
Diabetes decompensation	1 (0.4%)
Palpitation	1 (0.4%)

DISCUSSION

WE FOUND THAT in Japanese patients with G1H HCV-related LC, the likelihood of achieving SVR with IFN plus ribavirin combination therapy was influ-

Table 7 Factors associated with sustained virological response in the patients with G1H and TT allele of IL28B rs8099917 ($n = 133$)

Factors	SVR (+) ($n = 36$)	SVR (−) ($n = 97$)	*P*-value	Multivariate analyses		
				Odds ratio	95% CI	*P*-value
Platelets (10^4/mm^3)	14.5 ± 11.5	10.6 ± 4.2	0.024	1.08	1.01–1.18	0.047
Albumin (g/dL)	3.92 ± 0.50	3.69 ± 0.46	0.018	2.78	1.14–7.42	0.031
Treatment duration, over 48 weeks	$n = 27$ (75%)	$n = 51$ (52.6%)	0.023	2.53	1.07–6.49	0.042

P-values were obtained by logistic regression model.
CI, confidence interval; G1H, genotype 1 with high viral load; IL28B, interleukin 28B; SVR, sustained virological response.

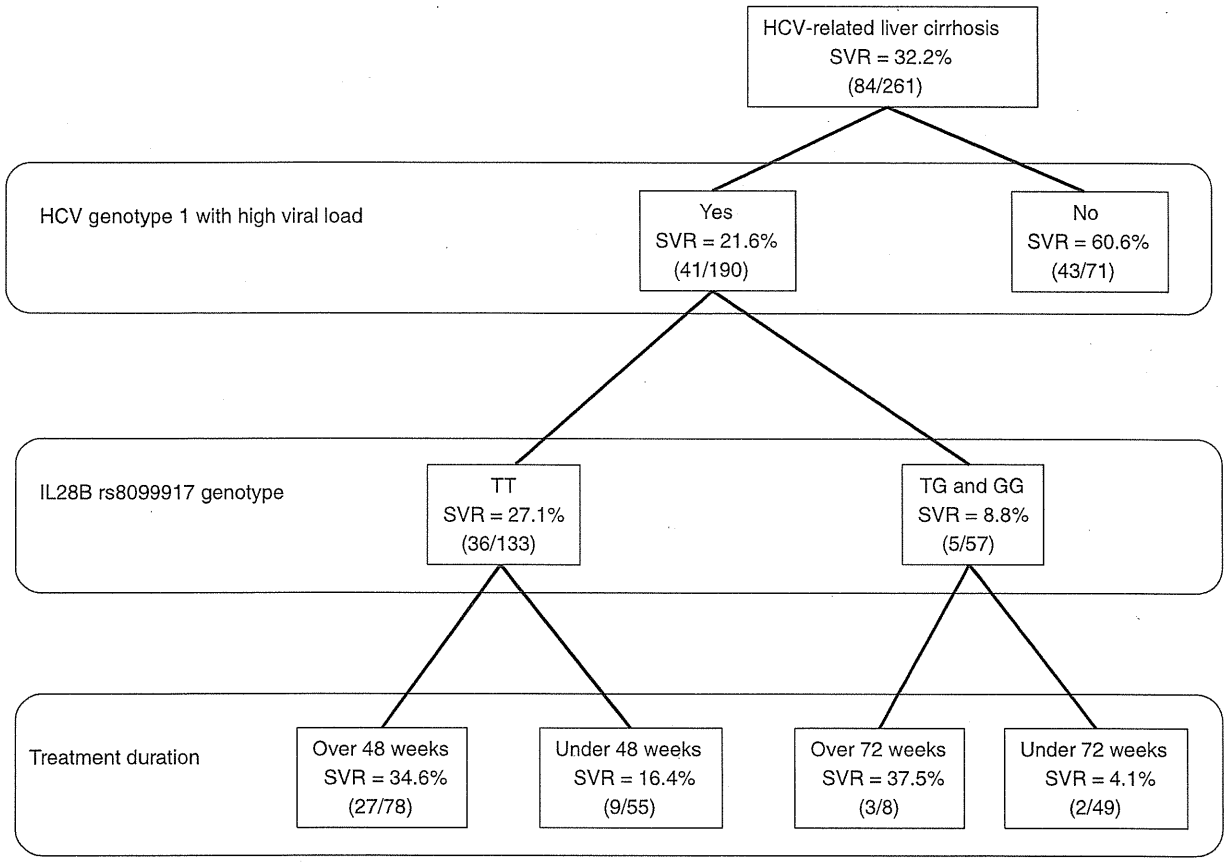

Figure 2 SVR in HCV-related liver cirrhosis patients treated with interferon plus ribavirin. In patients with G1H and the IL28B TT allele, the SVR rate of those who were treated for over 48 weeks was significantly higher than those treated for less than 48 weeks ($P = 0.042$). In patients with G1H and IL28B TG/GG, the SVR rate of patients treated for over 72 weeks was significantly higher than those treated for less than 72 weeks ($P = 0.010$). G1H, genotype 1 with high viral load; HCV, hepatitis C virus ; IL28B, interleukin 28B rs8099917; SVR, sustained virological response.

enced by a polymorphism at IL28B rs8099917. In contrast, SVR rates in non-G1H were higher than those in G1H, irrespective of IL28B genotype. This is the first report to demonstrate that an IL28B polymorphism can influence SVR rate in patients treated with IFN plus ribavirin combination therapy for G1H HCV-related LC. These results suggest that HCV genotypes, viral load and IL28B polymorphism should be taken into when determining antiviral therapy for HCV-related LC. In patients with HCV-related LC, IL28B genotyping may be a useful tool to determine the best antiviral therapy.

Recently, host genetic variation near the IL28B on chromosome 19, which encodes IFN-λ-3, have been shown to be associated with SVR to PEG IFN plus ribavirin in patients infected with HCV genotype 1.[11–13] Although some investigators have shown that IL28B polymorphisms are associated with a favorable response to treatment in patients with non-1 genotype infection, the association between the variants in IL28B and SVR in non-1 genotype-infected patients remains controversial.[19–25] IL28B polymorphisms are also a strong predictive factor for spontaneous HCV clearance.[26,27] However, the precise mechanism associated with the action of IL28B polymorphisms has not been fully elucidated.

Pegylated IFN plus ribavirin combination therapy has become the standard of care treatment for chronic HCV infection. The SVR rates range 42–46% in patients with HCV genotype 1 or 4 infection and 76–82% in patients with HCV genotype 2 or 3 infection, respectively.[9,28,29] However, in patients with HCV-related LC the SVR rate is even lower than in non-LC patients, reflecting reduced