provement in fibrosis. Genotypic resistance to entecavir ıerged in 31 patients for a 3-year cumulative resistance ɔbability of 35.9%. Entecavir was generally well tolerated ring ETV-060, with no on-treatment ALT flares.
ɔnclusions Long-term entecavir treatment of lamivuıe-refractory CHB resulted in virologic suppression, ALT rmalization, and improvements in liver histology. Resisıce was consistent with that observed in worldwide ıdies.

ɛywords Japanese · Chronic hepatitis B · Entecavir · mivudine refractory · Lamivudine resistant

troduction

ıronic hepatitis B (CHB) infection is a global public health ɔblem that is estimated to cause between 500,000 and 1.2 llion deaths annually [1–3]. Three-quarters of all chroniılly infected individuals live in the Asia–Pacific region, ıere hepatitis B virus (HBV) is the leading cause of chronic patitis, cirrhosis, and hepatocellular carcinoma (HCC) [4]. Japan, the prevalence of HBV infection was estimated to 0.8% in 2000, and the vast majority of individuals are ɛected with HBV genotype C [4–6]. Genotype C virus has en associated with high rates of progression to the comcations of CHB, including cirrhosis and HCC [7–11]. In dition to genotype, the level of HBV DNA in the serum is ongly associated with liver disease progression [12, 13]. rsistently detectable and elevated viral loads predict the ghest risk of progression to cirrhosis and HCC [12–14]. ppression of HBV replication with antiviral therapy may luce the risk of complications and improve the long-term tcomes of CHB patients [15].

Lamivudine has been widely used for the treatment of HB since its development and initial approval 10 years o [16, 17]. Lamivudine has demonstrated efficacy and ıg-term safety and was shown to result in histologic provement when administered for up to 3 years [16, 18,]. However, resistance to lamivudine emerges at a rate of proximately 20% per year and is found in approximately % of patients after 4 years of therapy [20, 21]. The ıergence of lamivudine resistance may be associated th increases in HBV DNA and alanine aminotransferase LT) levels, and loss of histologic response [16, 18, 22]. patients with cirrhosis, lamivudine resistance may lead hepatic decompensation and HCC [15, 23, 24]. Recently blished CHB treatment guidelines no longer recommend nivudine as first-line therapy for treatment-naive patients cause of the problems that resistance introduces in the ınagement of individual patients and the negative impact ıt lamivudine resistance has on the subsequent use of ıer antivirals [25].

Entecavir is a guanosine nucleoside analog that has demonstrated efficacy against nucleoside-naive and lamivudine-refractory CHB [26–29]. In global clinical studies, patients with lamivudine-refractory CHB treated with entecavir 1 mg daily for 48 weeks experienced reduction in HBV DNA levels of more than 5 log copies/mL and improvements in hepatic necroinflammation and fibrosis [28, 29]. Treatment for up to 96 weeks resulted in continued improvement of virologic, biochemical, and serologic end points [30]. In contrast to the nucleoside-naive population, emergence of resistance to entecavir occurred more frequently in the lamivudine-refractory population [30, 31]. To date, there are limited data on the efficacy of entecavir treatment beyond 96 weeks in the lamivudine-refractory patient population. A phase II study in Japan (ETV-052) demonstrated the efficacy and safety of entecavir in Japanese patients who were refractory to lamivudine therapy [32]. Immediately following completion of treatment in study ETV-052, patients were eligible to enroll in rollover study ETV-060 and receive entecavir 1 mg daily for up to 96 weeks. We present efficacy, safety, and resistance results for all patients treated in ETV-052 who rolled over into study ETV-060 for a total entecavir treatment time of up to 3 years (148 weeks). A subset of this cohort received the recommended dose of entecavir (1 mg daily) continuously from ETV-052 baseline, and results for this subset are also reported.

Materials and methods

Study design

Study ETV-060 was a long-term rollover study designed to provide open-label entecavir to lamivudine-refractory patients who completed treatment in the phase II study ETV-052 in Japan. In study ETV-052, 84 patients were randomized 1:1 to entecavir 0.5 mg ($n = 41$) or 1 mg ($n = 43$) daily for 52 weeks [32]. At baseline in this study, all patients had detectable lamivudine-resistance substitutions. Patients who completed 52 weeks of dosing in ETV-052 could enroll in ETV-060 and receive entecavir 1.0 mg daily in an open-label fashion. After completing 96 weeks of treatment in study ETV-060, patients could discontinue therapy or were eligible to receive commercially available entecavir that was approved by Japanese health authorities while ETV-060 was ongoing. The current analysis reports results for patients who completed ETV-052 and were subsequently treated in ETV-060 ($n = 82$) for a total entecavir treatment time (ETV-052 plus ETV-060) of up to 148 weeks. This cohort is termed the *lamivudine-refractory, long-term treatment cohort* (Fig. 1).

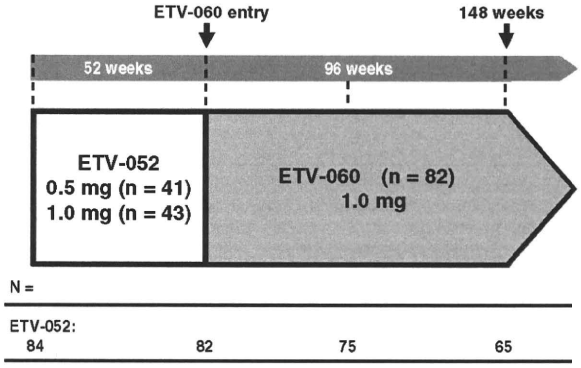

Fig. 1 Lamivudine-refractory, long-term treatment cohort. Eighty-two patients completed 52 weeks of treatment in study ETV-052 and entered rollover study ETV-060, with no interruption or gap in treatment. Sixty-five patients remained on treatment (entecavir 1.0 mg daily) through 96 weeks in study ETV-060, for a total entecavir treatment time of 148 weeks

During study ETV-060, clinical and laboratory measurements (serum chemistries, hematology, prothrombin time/international normalized ratio, and urinalysis) were assessed at baseline, weeks 2 and 4, and every 4 weeks thereafter throughout the dosing period. HBV DNA by PCR and HBV serologies were assayed at baseline, weeks 12 and 24, and subsequently every 24 weeks until week 96 or end of dosing. Liver biopsy specimens were obtained and scored for all patients at baseline and end (48 weeks) of study ETV-052, and repeat biopsy specimens were obtained at week 96 of study ETV-060 (148 weeks total entecavir treatment time) for patients who consented. Biopsy specimens were evaluated using the Knodell necroinflammatory and fibrosis scores and the corresponding New Inuyama classifications [33, 34].

Written informed consent was obtained from all patients, and the study was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice Guidelines, and Articles/Notifications of the Ministry of Health and Labor in Japan.

Patients

The inclusion criteria for study ETV-052 have been fully described elsewhere [32]. Eligible patients were adults with CHB infection and either evidence of active viral replication (HBV DNA $\geq 10^5$ copies/mL) despite at least 24 weeks of lamivudine therapy that was ongoing at the time of randomization or documented evidence of infection with HBV expressing lamivudine-resistance mutations. Patients could be hepatitis B e antigen (HBeAg)-positive or -negative and were required to have elevated levels of ALT [(1.3–10) × upper limit of normal (ULN)] and compensated liver disease. Exclusion criteria included coinfection with hepatitis C virus, hepatitis D virus, or human immunodeficiency virus; other forms of liver disease; therapy with any anti-HBV medication other than lamivudine within 24 weeks prior to randomization; and more than 12 weeks of therapy with a nucleoside or nucleotide analog (other than lamivudine) with activity against HBV. Pregnant and breast-feeding women were also excluded. All patients who completed 52 weeks of dosing in study ETV-052 were eligible to enroll in study ETV-060.

Efficacy and safety end points

Efficacy end points included the proportion of patients who achieved undetectable HBV DNA by PCR assay (<400 copies/mL), the proportion achieving ALT normalization (ALT $\leq$ 1.0 × ULN) among those with abnormal ALT at baseline, and the proportion with HBeAg loss and HBe seroconversion among those who were HBeAg-positive at baseline. Histologic results are presented for the cohort of patients who received entecavir 1 mg daily from phase II baseline and had evaluable liver biopsy pairs. Histologic improvement was defined as a $\geq$2-point decrease in the Knodell necroinflammatory score and no worsening of fibrosis (worsening: $\geq$1-point increase in the Knodell fibrosis score). Improvement in fibrosis was defined as a $\geq$1-point decrease in the Knodell fibrosis score. Histologic results were also assessed by the New Inuyama classification [34].

Safety analyses included the incidence of adverse events, serious adverse events, laboratory abnormalities, and discontinuations due to adverse events of treatment during study ETV-060, including results for patients treated beyond 96 weeks. ALT flare was defined as an on-treatment ALT measurement of more than 2 × baseline and more than 10 × ULN.

Resistance assessment

Genotypic analysis was performed on serum samples from all patients at baseline of study ETV-052 for evidence of the lamivudine-resistance substitution M204V/I in the HBV polymerase/reverse transcriptase. During study ETV-052, genotypic analysis to detect substitutions associated with entecavir resistance (at residues L180, T184, S202, M204, or M250 in the HBV polymerase/reverse transcriptase) was performed for patients with virologic breakthrough, defined as an increase in HBV DNA of $\geq$1 $\log_{10}$ copies/mL from nadir in two consecutive measurements or the last on-treatment measurement. During study ETV-060, serum samples were subjected to genotypic analysis to detect substitutions associated with entecavir resistance for patients who had HBV DNA of more than 400 copies/mL at week 100 or 148 (from study

ETV-052 baseline), or at the end of treatment (for patients who discontinued prior to week 148), and for patients who experienced virologic breakthrough.

Assay methods

All clinical laboratory tests, including HBV DNA levels, HBV serologies, and genotypic analyses, were performed at a central laboratory designated by the sponsor (SRL, Inc., Tokyo, Japan). Serum HBV DNA levels were determined by the Roche Amplicor™ PCR assay (limit of quantification = 400 copies/mL; Roche Diagnostics K.K., Tokyo, Japan). Lamivudine-resistance substitutions were identified using a PCR enzyme-linked minisequence assay (Medical & Biological Laboratories Co., Ltd., Aichi, Japan). On-treatment resistance testing was carried out by extraction of HBV DNA followed by PCR amplification and sequencing of codons 1–344 of the reverse transcriptase encoding region.

Statistical analysis

Descriptive summaries were performed. Analyses of efficacy and safety end points were based on patients who received at least one dose of study medication in study ETV-060. For binary end points, patients with missing on-treatment measurements were treated as missing (non-completer = missing analysis). Parameters represented by continuous variables were summarized by means and standard errors. Analyses of HBV DNA as a continuous parameter were applied after $\log_{10}$ transformation.

Results

Study population

Eighty-four patients were treated with entecavir in phase II study ETV-052, and 82 patients entered ETV-060, constituting the lamivudine-refractory, long-term treatment cohort (Fig. 1). Seventeen patients discontinued treatment during ETV-060 for the following reasons: adverse event ($n = 8$), protocol violation ($n = 1$), loss to follow-up ($n = 1$), and insufficient effect in the judgment of the investigator ($n = 7$). Sixty-five patients completed 96 weeks of treatment in ETV-060 for a total of 148 weeks of entecavir from ETV-052 baseline through ETV-060 (Fig. 1). Baseline (pretreatment) demographics and disease characteristics of this cohort ($n = 82$) are shown in Table 1. Eighty-seven percent (71/82) of patients were men, and mean age was 44 years. Mean HBV DNA level was 7.69 $\log_{10}$ copies/mL, mean ALT level was 135 IU/L, and 76% (62/82) of patients were HBeAg positive. All patients had documented lamivudine-resistance substitutions at M204. Ninety-four percent (77/82) of patients were infected with HBV genotype C.

Table 1 Pretreatment baseline demographics and disease characteristics of the lamivudine-refractory long-term treatment cohort ($n = 82$)

Characteristic	ETV-060 Etecavir 1.0 mg, $n = 82$
Male, *n* (%)	71 (86.6)
Age, years, mean	43
Weight, kg, mean (±SD)	66.81 (10.58)
HBV DNA, mean $\log_{10}$ copies/mL (±SD)	7.69 (0.91)
HBeAg-positive, *n* (%)	62 (75.6)
ALT, IU/L, mean (±SD)	134.7 (111.3)
ALT > 1.0 × ULN, *n* (%)	78 (95.1)
M204V/I mutation present, *n* (%)	82 (100)
HBV genotype, *n* (%)	
A	1 (1.22)
B	2 (2.44)
C	77 (94)
Others	2 (2.44)

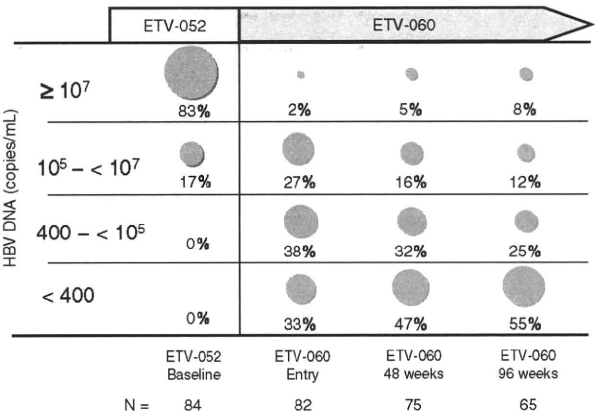

Fig. 2 Distribution of HBV DNA over time in the lamivudine-refractory, long-term treatment cohort. The proportion of patients with HBV DNA of >400 copies/mL increased through ETV-060 week 96 (148 weeks of total entecavir treatment time)

Virologic response

HBV DNA was suppressed and decreased rapidly during phase II study ETV-052 [32]. For the 82 patients who entered ETV-060 after completing ETV-052, mean HBV DNA level decreased from 7.69 $\log_{10}$ copies/mL at pretreatment baseline to 3.99 $\log_{10}$ copies/mL at ETV-060 entry (after 52 weeks of entecavir treatment). HBV DNA was further suppressed during 96 weeks of treatment in ETV-060. At baseline of study ETV-060, 33% of patients (27/82) had HBV DNA of >400 copies/mL (Fig. 2), and

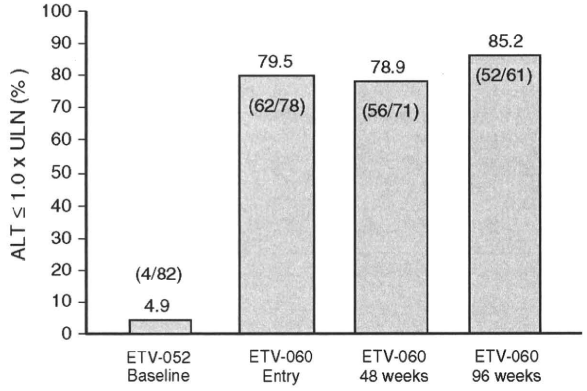

Fig. 3 Proportions of patients with normal ALT (ALT $\leq$ 1.0 $\times$ ULN) over time in the lamivudine-refractory, long-term treatment cohort. Seventy-eight patients had abnormal ALT (ALT > 1.0 $\times$ ULN) at pretreatment baseline. At week 96 of study ETV-060, patients had received a total of 148 weeks of entecavir therapy

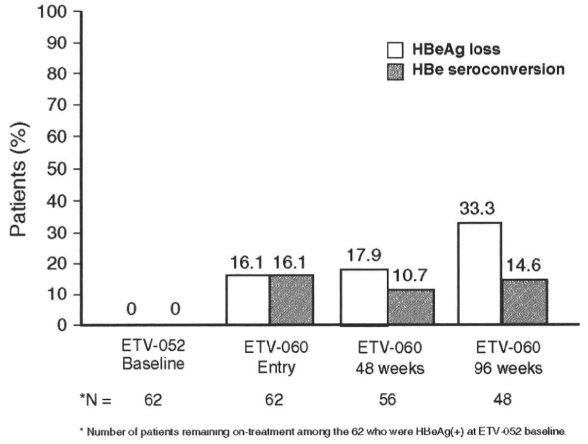

Fig. 4 Proportions of patients with HBeAg loss and HBe seroconversion over time in the lamivudine-refractory, long-term treatment cohort. Sixty-two patients were HBeAg positive at pretreatment baseline. At week 96 of study ETV-060, patients had received a total of 148 weeks of entecavir therapy

this proportion increased to 55% (36/65) by week 96 of ETV-060 (148 weeks total entecavir treatment time). Of the 17 patients who discontinued treatment during ETV-060, one patient had HBV DNA of >400 copies/mL at the last on-treatment measurement.

Biochemical response

At pretreatment baseline, 95.1% (78/82) of patients had abnormal ALT (ALT > 1.0 $\times$ ULN; Table 1; Fig. 3). After 52 weeks of treatment in ETV-052, 79.5% (62/78) of patients had normalized ALT. After 96 weeks of further treatment in ETV-060 (148 weeks total entecavir treatment time), ALT had normalized in 85.2% (52/61) of patients.

Serologic response

Sixty-two patients (76%) were HBeAg-positive at pretreatment baseline (Table 1; Fig. 4). At ETV-060 entry, 16.1% (10/62) of these patients had achieved HBe seroconversion and the same number had lost HBeAg (Fig. 4). After 96 weeks in ETV-060 (148 weeks total entecavir treatment time), 33.3% of patients (16/48) had lost HBeAg and 14.6% (7/48) had undergone HBe seroconversion.

Resistance analysis

No substitutions associated with entecavir resistance emerged during study ETV-052 [32]. Eighty-one of 82 patients were monitored for resistance from ETV-052 baseline through to the end of treatment in ETV-060 (1 patient refused consent for resistance testing). Thirty-one patients developed genotypic resistance to entecavir during the second or third year of treatment, of whom 21 experienced virologic breakthrough. The 3-year cumulative probability of resistance was 35.9% [35].

Safety

Mean exposure to entecavir during study ETV-060 was 101.3 weeks (range 7.1–148). All patients experienced at least one adverse event, and 11% (9/82) experienced serious adverse events (Table 2). One patient was diagnosed with HCC at week 57 of ETV-060. Eight patients (9.8%) discontinued treatment during ETV-060 because of adverse events, such as increased ALT, virologic breakthrough, and genotypic resistance emergence. Five of these eight patients had received entecavir 0.5 mg daily during phase II study ETV-052, and three received entecavir 1 mg from phase II baseline. There were no ALT flares during ETV-060, and no deaths were reported during the study.

Entecavir 1-mg cohort

A subset of 42 patients (42/82) received the recommended 1-mg dose of entecavir for lamivudine-refractory CHB from phase II baseline through to the end of treatment in study ETV-060. In this subset, among patients with available samples, 54% (19/35) had HBV DNA of >400 copies/mL, 84% (27/32) had ALT of $\geq$1 $\times$ ULN, and 15% (4/27) achieved HBe seroconversion after 3 years of continuous treatment with entecavir 1 mg daily. Genotypic resistance emerged in 13 patients in this cohort, and 9 of 13 patients experienced virologic breakthrough. The cumulative 3-year probability of resistance was 30.4%.

Table 2 Summary of safety during ETV-060 in the lamivudine-refractory long-term treatment cohort

	n (%) ETV-060 Entecavir 1.0 mg *n* = 82 (%)
Any adverse event	82 (100)
Clinical adverse events	78 (95.1)
Clinical serious adverse events	6 (7.3)
Grade 3–4 clinical adverse events	2 (2.4)
Most frequent clinical adverse events	
Nasopharyngitis	57 (69.5)
Headache	21 (25.6)
Diarrhea	12 (14.6)
Back pain	8 (9.8)
Laboratory adverse events	77 (93.9)
Laboratory serious adverse events	3 (3.7)
Grade 3–4 laboratory adverse events	15 (18.3)
ALT increased	24 (29.3)
ALT flare[a]	0
Discontinuations due to adverse events	8 (9.8)
Deaths	0

[a] ALT > 2 × baseline and >10 × ULN

Sixteen (16/42) patients in the 1-mg cohort had paired evaluable liver biopsies from three time points: pretreatment (phase II) baseline, week 48, and week 148 total entecavir treatment time (ETV-060, week 96). Of these, 81% (13/16) demonstrated histologic improvement from baseline through week 148. The mean Knodell necroinflammatory score improved from 6.06 at baseline to 1.44 at week 148, and all patients (16/16) exhibited minimal necroinflammation (a Knodell necroinflammatory score of ≤3 points) at week 148 (Fig. 5a). Knodell fibrosis scores improved in 38% (6/16) of patients from baseline through week 148, and the mean Knodell fibrosis score decreased from 2.44 at baseline to 1.94 at week 148 (Fig. 5b). Liver biopsy assessments using the New Inuyama classification system confirmed the results obtained using the Knodell classification system (data not shown).

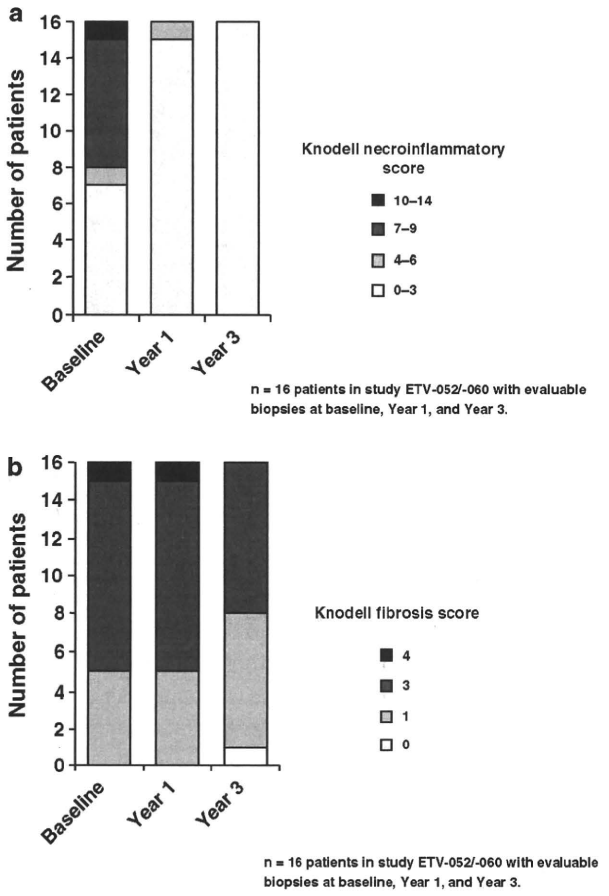

Fig. 5 Distribution of Knodell necroinflammatory scores (**a**) and Knodell fibrosis scores (**b**) at baseline, year 1 (48 weeks), and year 3 (148 weeks) for the 16 patients who had evaluable liver biopsies at all 3 time points

Discussion

This report describes the results of 3 years of continuous entecavir therapy in a lamivudine-refractory patient population. All patients in the lamivudine-refractory, long-term treatment cohort had highly elevated levels of HBV DNA with documented lamivudine-resistance mutations at baseline, and 94% were infected with HBV genotype C. This represents a population with potentially poor long-term outcomes. Patients with lamivudine-resistant HBV may have cross-resistance to other antivirals, and genotype C infection is associated with low rates of HBe seroconversion and high rates of liver disease progression [7, 25, 36]. These results show that entecavir therapy for up to 3 years for this population resulted in durable HBV DNA suppression and ALT normalization. More than 50% of patients in the cohort achieved undetectable HBV DNA and almost 90% normalized ALT by year 3. Similar levels of HBV DNA suppression and ALT normalization were observed for the subset of patients who received entecavir 1 mg daily throughout the treatment period. Among patients with liver biopsies from three time points (all of whom received the recommended 1-mg dose of entecavir from phase II baseline), substantial improvements in liver histology were observed: more than 80% of patients demonstrated histologic improvement at year +++3 and slow improvements in fibrosis were observed in 38% of patients.

In previously published results of a multinational clinical trial, entecavir demonstrated potent inhibition of viral

replication in HBeAg-positive, lamivudine-refractory patients infected with a variety of HBV genotypes (A–D) [28, 30]. In that trial, after 48 weeks of treatment with entecavir 1 mg daily, the mean change from baseline in HBV DNA was -5.11 $\log_{10}$ copies/mL, and 19% of patients achieved HBV DNA of >300 copies/mL. Among patients who continued to a second year of entecavir therapy, the mean change from baseline in HBV DNA increased to -5.9 $\log_{10}$ copies/mL, and 40% of patients achieved HBV DNA of >300 copies/mL. In the current study in Japanese patients, 54% achieved HBV DNA of >400 copies/mL. The higher proportion of Japanese patients suppressing HBV DNA to below the PCR limit of quantification in the current study likely reflects the effect of an additional year of entecavir therapy, as well as the lower baseline HBV DNA (7.69 $\log_{10}$ vs. 9.59 $\log_{10}$ copies/mL in the multinational study). The relatively low rate of HBe seroconversion observed in this study (15%) may be related to infection with genotype C virus. In studies in Japan and elsewhere in Asia, HBV genotype C has been associated with lower seroconversion rates than with other HBV genotypes [7, 36–38].

Achieving and maintaining HBV DNA suppression is a principal goal of CHB therapy [25, 39]. Data from prospective long-term studies have shown that elevated HBV DNA levels are associated with the development of long-term complications including cirrhosis and HCC [12–14]. Other research has correlated durable HBV DNA suppression with improved liver histology among antiviral-treated patients [19, 40]. Liaw et al. [15] showed that lamivudine therapy benefits CHB patients with advanced liver disease by reducing the risk of liver disease progression, including the development of HCC. In the present study, the reduction in hepatic necroinflammation and fibrosis observed in a subset of patients through 3 years, along with the durable virologic suppression observed in the larger cohort, suggests that entecavir helps halt or reverse liver disease progression that can lead to poor long-term outcomes.

The emergence of lamivudine resistance can lead to serious clinical consequences, including elevated levels of HBV DNA, exacerbations of hepatitis, and hepatic decompensation [18, 22, 23, 41]. While early studies of patients with lamivudine-resistant HBV suggested that switching to adefovir was efficacious, subsequent work demonstrated the rapid emergence of adefovir resistance in this patient population [42–44]. The emergence of adefovir resistance in this setting can be associated with viral rebound and hepatic decompensation [45]. Adding adefovir to ongoing lamivudine for patients who have developed lamivudine resistance has been recommended as a strategy to reduce the subsequent emergence of adefovir resistance [25, 46]. This strategy is most efficacious in patients with low HBV DNA levels and requires continued resistance surveillance [47, 48]. Studies evaluating the combination of entecavir with adefovir in lamivudine-resistant patients are currently in progress.

The rate of genotypic resistance to entecavir reported here is consistent with the rate that has been observed in multinational populations of lamivudine-refractory patients [49]. In nucleoside-naive patients, emergence of entecavir resistance is rare because of entecavir's potent viral load reduction and high genetic barrier to resistance [49, 50]. Substitutions at M204 ± L180 were detected at baseline for all patients described in this report and have been shown in previous studies to reduce in vitro susceptibility to entecavir by approximately eightfold [51]. Resistance to entecavir requires the presence of the rtM204V/I lamivudine-resistance substitution plus at least one additional amino acid substitution at rtT184, rtS202, or rtM250. In the current study, for the subset of patients who received entecavir 1 mg daily throughout the treatment period, the cumulative rate of entecavir resistance was 30% through 3 years. This is consistent with the rate observed in the entire lamivudine-refractory, long-term treatment cohort and in multinational studies of lamivudine-refractory patients through 3 years (36%) [49]. Combining entecavir with an antiviral with a different resistance profile, such as tenofovir or adefovir, may result in less frequent resistance emergence.

Entecavir was well tolerated during treatment in study ETV-052, with no discontinuations due to adverse events and three early on-treatment flares that were transient and associated with declining levels of HBV DNA [32]. Throughout the extended treatment period during ETV-060, entecavir continued to be well tolerated with relatively few discontinuations and no ALT flares observed. There were no deaths during the study, and one patient was diagnosed with HCC at week 57 of ETV-060. The extent to which long-term treatment with entecavir may reduce development of HCC in CHB patients remains under investigation.

In summary, these results show that treatment with entecavir for up to 3 years in lamivudine-refractory CHB results in continued benefit beyond the first year, including durable HBV DNA suppression and progressive improvements in liver histology, with a resistance profile consistent with that observed in other studies. Entecavir at the recommended dose of 1 mg daily is an option for patients with lamivudine-refractory CHB. Additional research evaluating the combination of entecavir plus adefovir or tenofovir in this patient population is ongoing.

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ERRATUM

Erratum to: Efficacy and resistance of entecavir following 3 years of treatment of Japanese patients with lamivudine-refractory chronic hepatitis B

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Page 414
Abstract
Results

After 96 weeks in ETV-060 (148 weeks total entecavir treatment time), 55% (36/65) of patients had hepatitis B virus (HBV) DNA of <400 copies/mL, 85% (52/61) had alanine aminotransferase (ALT) of ≤1 × upper limit of normal (ULN), and 14.6% (7/48) achieved HBe seroconversion.

A subset of 42 patients received entecavir 1 mg from phase II baseline through 148 weeks: 54% (19/35) had HBV DNA of <400 copies/mL, 84% (27/32) had ALT of ≤1 × ULN, and 15% (4/27) achieved HBe seroconversion.

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Fig. 2 Distribution of HBV DNA over time in the lamivudine refractory, long-term treatment cohort. The proportion of patients with HBV DNA of <400 copies/mL increased through ETV-060 week 96 (148 weeks of total entecavir treatment time).

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Page 417, Virological response, line 9

HBV DNA was further suppressed during 96 weeks of treatment in ETV-060. At baseline of study ETV-060, 33% (27/82) of patients had HBV DNA of <400 copies/mL (Fig. 2).

Page 418, Entecavir 1-mg cohort, lines 5 and 6

In this subset, among patients with available samples, 54% (19/35) had HBV DNA of <400 copies/mL, 84% (27/32) had ALT of $\leq 1 \times$ ULN, and 15% (4/27) achieved HBe seroconversion after 3 years of continuous treatment with entecavir 1 mg daily.

Page 420, lines 6, 10, 12

In that trial, after 48 weeks of treatment with entecavir 1 mg daily, the mean change from baseline in HBV DNA was -5.11 $\log_{10}$ copies/mL, and 19% of patients achieved HBV DNA of <300 copies/mL. Among patients who continued to a second year of entecavir therapy, the mean change from baseline in HBV DNA increased to -5.9 $\log_{10}$ copies/mL, and 40% of patients achieved HBV DNA of <300 copies/mL. In the current study in Japanese patients, 54% achieved HBV DNA of <400 copies/mL.

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Review Article

Predictors of Virological Response to a Combination Therapy with Pegylated Interferon Plus Ribavirin Including Virus and Host Factors

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A combination therapy with pegylated interferon (PEG-IFN) plus ribavirin (RBV) has made it possible to achieve a sustained virological response (SVR) of 50% in refractory cases with genotype 1b and high levels of plasma HCVRNA. Several factors including virus mutation and host factors such as age, gender, fibrosis of the liver, lipid metabolism, innate immunity, and single nucleotide polymorphism (SNPs) are reported to be correlated to therapeutic effects. However, it is difficult to determine which factor is the most important predictor for an individual patient. Data mining analysis is useful for combining all these together to predict the therapeutic effects. It is important to analyze blood tests and to predict therapeutic effects prior to initiating treatment. Since new anti-HCV agents are under development, it will be necessary in the future to select the patients who have a high possibility of achieving SVR if treatment is performed with standard regimen.

1. Progress in Virological Response in the Difficult-to-Treat Patients with Genotype 1 Hepatitis C Virus (HCV) Infection and Factors Correlated to the Efficacy

Recently, the average age of the patients with chronic hepatitis C has been increasing in Japan. Incidence of hepatocellular carcinoma (HCC) in the elderly patients with chronic hepatitis C (65 years or older) has demonstrated to be higher than younger ones when adjusted by the stage of hepatic fibrosis [1]. In Japan, refractory cases with genotype 1b and high HCVRNA levels are seen in as high as 70 percent of chronic hepatitis C patients. The outcome of conventional IFN monotherapy has been an SVR response of 3%–5% after 6 months of treatment in genotype 1b and high HCVRNA patients [2, 3], and virus mutation such as interferon sensitivity-determining region (ISDR) is shown to be correlated with the virological response [2]. The association of ISDR mutations and virological response to IFN monotherapy was denied in an Italian study [4]; however, it was confirmed by a Chinese study [5] and an international meta-analysis [6].

However, pegylated IFN (PEG-IFN) extends the duration of therapy and reduces adverse effects, and for this reason, PEG-IFN has become the cornerstone of therapy. Furthermore, by the combination therapy with PEG-IFN and ribavirin (RBV), the rate of SVR has dramatically improved. Even in the patients with genotype 1b and high HCVRNA level, SVR rate reaches as high as 40%–50%, thereby improving the therapeutic effects both in Western countries [7, 8] and in Japan [9, 10].

It is important to predict the rate of achieving SVR in the individual patient, before initiating treatment. Both virus- and host-related elements have been reported as factors correlated to therapeutic effects of combination therapy [11–13]. A particular focus has been placed on virus mutations, age, gender, fibrosis of the liver, lipid metabolism, and degree of fatty metamorphosis of the liver.

Among these factors related to PEG-IFN and RBV, innate immunity has been shown to be correlated in virological

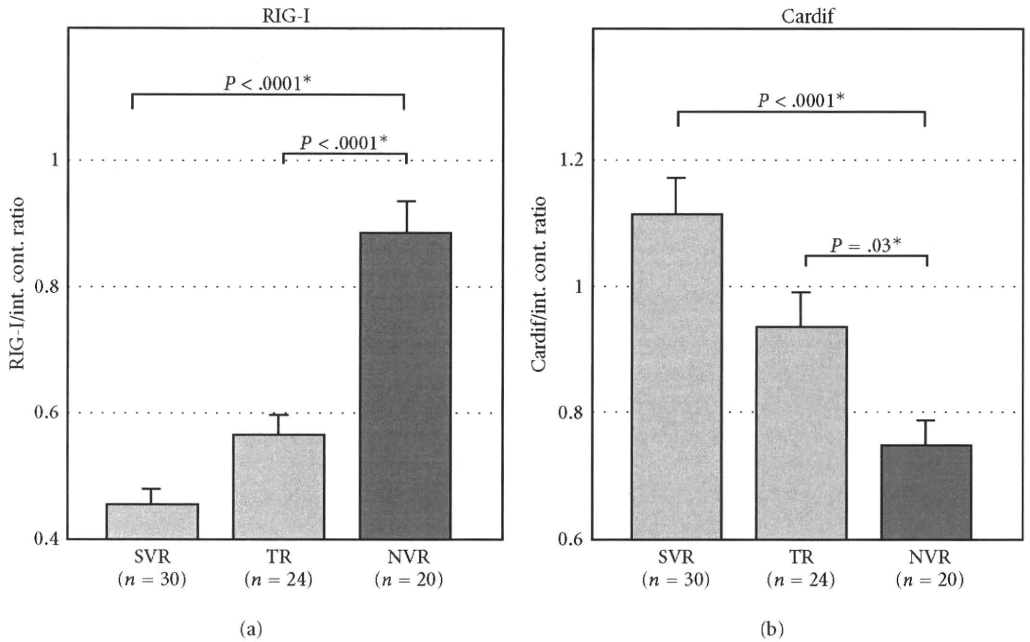

FIGURE 1: Expression of genes correlated to the intrahepatic innate immunity and virological response to PEG-IFN alpha-2b and RBV combination therapy. Open column indicates SVR ($n = 30$), gray column indicates TR ($n = 24$), and closed column indicates NVR ($n = 20$). Error bars indicate the standard error. The P values were analyzed by the Kruskal-Wallis test. Expression of Rig-I was significantly higher in NVR than in SVR patients, and Cardif expression was higher in SVR than in NVR. The figure was cited from [8].

response. Asahina et al. reported that liver biopsies were performed before the PEG-IFN and RBV combination therapy to examine the correlation between the gene expression involved in innate immunity and the therapeutic effects, and in the patients in whom RIG-I expression is high and the expression of Cardif, an adaptor gene, is low, it was found that there are many nonresponders (NVRs) in which HCVRNA does not become negative during the course of treatment [13]. It was therefore revealed that there are many NVRs among the patients in whom the ratio of RIG-I to Cardif in liver tissue is high and that this ratio is low in the SVR patients. Based on these findings, it has become clear that innate immunity is correlated to therapeutic effects (Figure 1).

Furthermore, it was recently discovered that a single nucleotide polymorphism (SNP) of the host gene IL28B is significantly involved in the therapeutic response to the PEG-IFN and RBV combination therapy [14, 15]. The possibility of becoming an NVR is high in cases of the minor allele carriers of IL28B. However, it is not possible to routinely measure an SNP of IL28B in the clinical setting. In this paper, factors which can actually be used in real clinical practice are discussed for the prediction of the efficacy of PEG-IFN and RBV combination therapy.

2. Amount of HCVRNA

In the patients with chronic hepatitis C, it is not possible to directly measure the amount of virus, and the amount of HCVRNA is measured instead. Currently, a real-time PCR method which has an advantage of wide range and high sensitivity is utilized, and measurements can be taken from a single blood sample of a very small amount, that is, 1.2 log copies/ml, to a very large amount, that is, 8 log copies/ml. This method has a higher level of sensitivity than the conventional Amplicor monitor test and can therefore detect HCVRNA even if only a very small amount exists in the plasma. If the amount of HCVRNA in plasma is less than the range of sensitivity of the real-time PCR method, it is recorded as undetectable level. If the indication is "less than 1.2 log copies/ml of HCVRNA", it means that a very small amount of HCVRNA exists in the plasma. Since the indication of the real-time PCR method is based on log counts, a decrease of 1.0 in the numerical value means that the amount of HCVRNA has decreased to 1/10. With the application of this real-time PCR method, it has become possible to measure amounts of HCVRNA up to 8 log copies/ml, and it has also become possible to predict the efficacy before treatment and to monitor appropriately the reactivity during the course of treatment. However, in the patients in whom a PEG-IFN and RBV combination therapy is performed, SVR can be acquired even when the amount of virus prior to the treatment is quite large. It is therefore difficult to predict the virological response solely from the amount of HCVRNA before starting the treatment. Once treatment has commenced, at what week HCVRNA becomes negative is important for the prediction of therapeutic effects, and this serves as a parameter for deciding the duration of treatment [16].

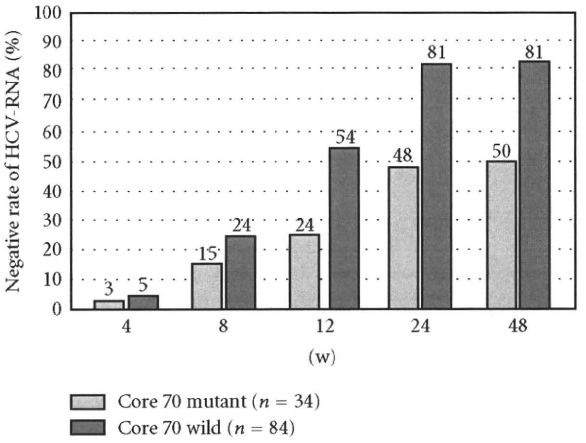

FIGURE 2: Comparison of aa70 mutations in the HCV core region and the rate of HCVRNA becoming negative during the course of treatment. Compared with the wild type, among the patients of aa70 mutations, there were fewer patients in whom HCVRNA had become negative during the course of treatment.

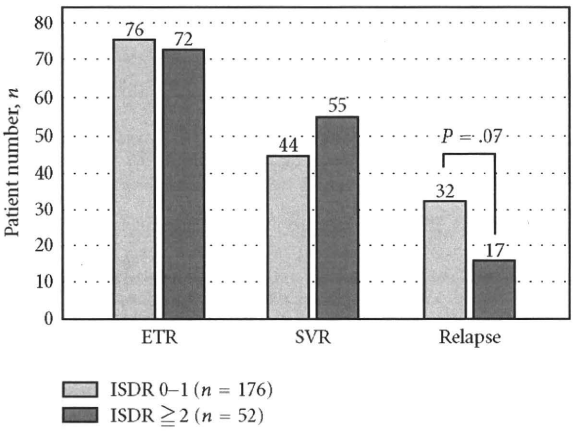

FIGURE 3: Number of ISDR substitutions and the comparison of virological response, SVR, and relapse at the end of the treatment. Compared with the patients with 2 or more sites of substitutions, the rate of SVR was lower and the rate of relapse was higher in the patients in whom there were fewer substitutions in ISDR, that is, 0 or 1 sites.

Measuring the rate of viral clearance from serum is helpful for predicting the likelihood of a response to PGEIFN and RBV and useful for determining the optimal duration of therapy if the patients start to receive the treatment [17]. In the AASLD practice guideline, response-guided therapy is highly recommended [18]. In two nationwide registration trials conducted in Japan [9, 10], the SVR rate was high, from 76% to 100%, in patients whose HCVRNA was cleared rapidly from serum by week 4 (rapid virological response; RVR), and 71% to 73% in patients who achieved undetectable HCVRNA from week 5 to week 12 (early virological response; EVR). In contrast, the SVR rate in patients with late clearance of HCVRNA from week 13 to week 24 was low at 29% to 36%. No patients without clearance of HCVRNA by week 24 achieved SVR.

The strategy of extending therapy in patients with delayed virological responses, defined as clearance of HCV-RNA between weeks 12 and 24, was evaluated in five studies [19–23]. These results cannot be compared directly with each other because of the heterogeneous study populations, differences in the baseline characteristics, and the different regimens utilized amongst them. Nevertheless, the results showed a trend toward a higher SVR rate by extending therapy from 48 to 72 weeks in patients with delayed virological response.

3. Viral Mutations in Core and NS5A Region

In the patients with genotype 1b HCV infection, the mutations in aa70 and aa91 in the core region have been shown to correlate with early virological response (EVR) during PEG-IFN and RBV combination treatment [11]. If aa70 in the core region is mutated to anything other than arginine and aa91 to anything other than leucine, it is difficult to achieve EVR, and it is thus highly possible that such cases will become nonresponders. The examination results at our institution including 292 patients with genotype 1b infection demonstrated that, in the cases with mutations in aa70 in the core region, the rate of HCVRNA becoming negative during the course of combination treatment was low compared to the wild type of aa70 (Figure 2). However, core aa70 mutation is shown to have quasispecies detectable by cloning, and 70Q clone was positively selected during combination treatment with PEGIFN and RBV [24].

Furthermore, Enomoto et al. reported that the patients with 4 or more amino acid mutations were observed in interferon sensitivity-determining region (ISDR) within NS5A region [2]; SVR rate is higher than 90% by IFN monotherapy, and SVR is less than 10% in the patients with no mutation in ISDR. It has also been reported that, in PEG-IFN and RBV combination therapy, the number of ISDR mutations is involved in the SVR [12].

We analyzed the relationship between virological response and ISDR mutations in the patients with genotype 1b infection treated by PEG-IFN alpha-2b and BRV combination therapy. In the patients with 0 or 1 ISDR mutation, even if the rate of HCVRNA becoming negative at the end of treatment was the same, the rate of SVR would be lower compared with the patients having 2 or more mutations (Figure 3). This demonstrates that there is a higher incidence of relapse after the end of treatment in the patients with 0 or 1 ISDR mutation.

Enomoto and Maekawa reported that mutations both in NS5A-ISDR (interferon sensitivity-determining region) and core 70Q substitution are associated with no early viral response during PEGIFN and RBV combination therapy [25]. Association of core aa70 substitution and mutations in NS5A region is confirmed to be associated with viral response by PEGIFN and RBV combination therapy in a Japanese multicenter cooperative study [26]. The number of

mutations in the interferon sensitivity-determining region was shown to be associated with the viral response to PEGIFN and RBV combination treatment not only in Japan [27], but also in Tunisia [28].

Recently, a consensus has been established that mutations in aa70 in the core region are important for the prediction of HCVRNA becoming negative during the early course of treatment, and the number of ISDR mutations is important for the prediction of relapse after the end of treatment.

4. Adherence

It has been confirmed that it is important to ensure 80% or more of the scheduled dose of both PEG-IFN and BBV in order to improve the rate of SVR, and together with the duration of treatment, the 80 · 80 · 80 rule has been established. However, Schiffman et al. recently reported that the dose of PEG-IFN in the initial stage of administration is important and that, if a sufficient dose of PEG-IFN is administered, then 60% or more of the RVB dose would be enough [29]. It is therefore of primary importance to ensure the dose of PEG-IFN.

In Japan, the average age of patients with chronic hepatitis C is increasing, and achieving good adherence is difficult in many patients. Consequently, the rate of SVR is low in elderly patients. How to improve the rate of SVR in elderly patients is an important issue. With regard to the dose of RBV, reducing the RBV dose based on the calculation of the total body clearance (CL/F) has been proposed to be useful for decreasing the discontinuation and improving the rate of SVR. Although there is no consensus on an appropriate dose of PEG-IFN in elderly patients, if the initial dose is set lower than the usual dose, discontinuation would be reduced. Thus, it is necessary to investigate whether such an initial dose would improve the rate of SVR.

Recently, the risk of hemolytic anemia was clearly demonstrated to correlate with ITAP gene SNP [30]. The predictive implication should be analyzed prospectively in clinical practice.

5. Host Factors

Zeuzem et al. described the factors related to the less response to interferon-based therapy, and he showed that several host factors such as older age, race, and obesity are responsible factors for the poor response to IFN [31]. Recently, insulin resistance which was examined by homeostasis model assessment index (HOMA-IR) was shown to be associated with a lower rate of SVR, and body mass index (BMI) was not identified as a significant factor for the poor response to PEGIFN and ribavirin combination therapy [32]. Insulin resistance was confirmed as a related factor to the nonresponse to interferon-based treatment [33]. However, Charlton et al. reported that obesity itself is an associated factor for decreased efficacy of interferon-based therapies, and they discussed the possible mechanism [34], and obesity was shown to be associated with the increased enhancement of suppressor of cytokine signaling (SOCS) family in the hepatocytes [35].

6. Data Mining Analysis

Both virus- and host-related factors are correlated to therapeutic effects of PEG-IFN and RBV. One important question is which of these factors should be focused on in order to predict the therapeutic effects in an individual patient. In addition, in each individual patient, the host and virological factors are different. It is therefore difficult to predict the virological response in each case before treatment. Furthermore, although it is important to predict the relapse rate when HCVRNA becomes within an undetectable level in an individual patient, prediction of the rate of SVR including virological and host factors and adherence to the treatment has never been carried out in an individual patient.

A data mining analysis is the process of analyzing a large amount of data by a computer in order to develop an algorithm. Conventional statics have been used to examine certain hypothesis. Data mining is superior in that it can set an algorithm, using a computer, based on a large amount of data without a hypothesis.

We therefore conducted at our institute a data mining analysis of the patients with genotype 1b infection having high levels of HCVRNA to whom a PEG-IFN alpha-2b and RBV combination therapy was administered to investigate whether by the 12th week after the commencement of treatment HCVRNA became negative (EVR) (Figure 4) [36]. The most important factor for the prediction of EVR was the steatosis of the liver: when steatosis was observed in 30% or more of hepatocytes, EVR was found to be difficult to achieve. In the patients in whom steatosis was not severe, the second most important factor was the serum LDL cholesterol value. While the rate of EVR was 57% in the patients in whom this value was 100 mg/ml or above, the rate of EVR was 32% in the patients in whom the LDL cholesterol was less than 100 mg/dl.

The higher the LDL cholesterol value, the earlier the HCVRNA became negative. Among the patients with low LDL cholesterol values, while the rate of EVR was 15% in patients 60 years of age or older, the rate was high in the patients under the age of 60 years old, that is, 49%. Among patients under the age of 60, the rate of EVR was low, that is, 31%, in patients with a blood glucose level of 120 mg/dl or above whereas EVR was achieved in 71% of the patients with a blood glucose level of less than 120 mg/dl (Figure 4).

On the other hand, in the patients with high LDL cholesterol values, the next most important factor was age. While the rate of EVR was 50% in patients 50 years of age or older, EVR was achieved in 77% of the patients under the age of 50. Among patients of 50 years of age or older, the next most important factor was the gamma GTP value. While the rate of EVR was 35% in the patients in whom gamma GTP was 40 IU/L or above, EVR was achieved in 60% of the patients where the value was less than 40 IU/L.

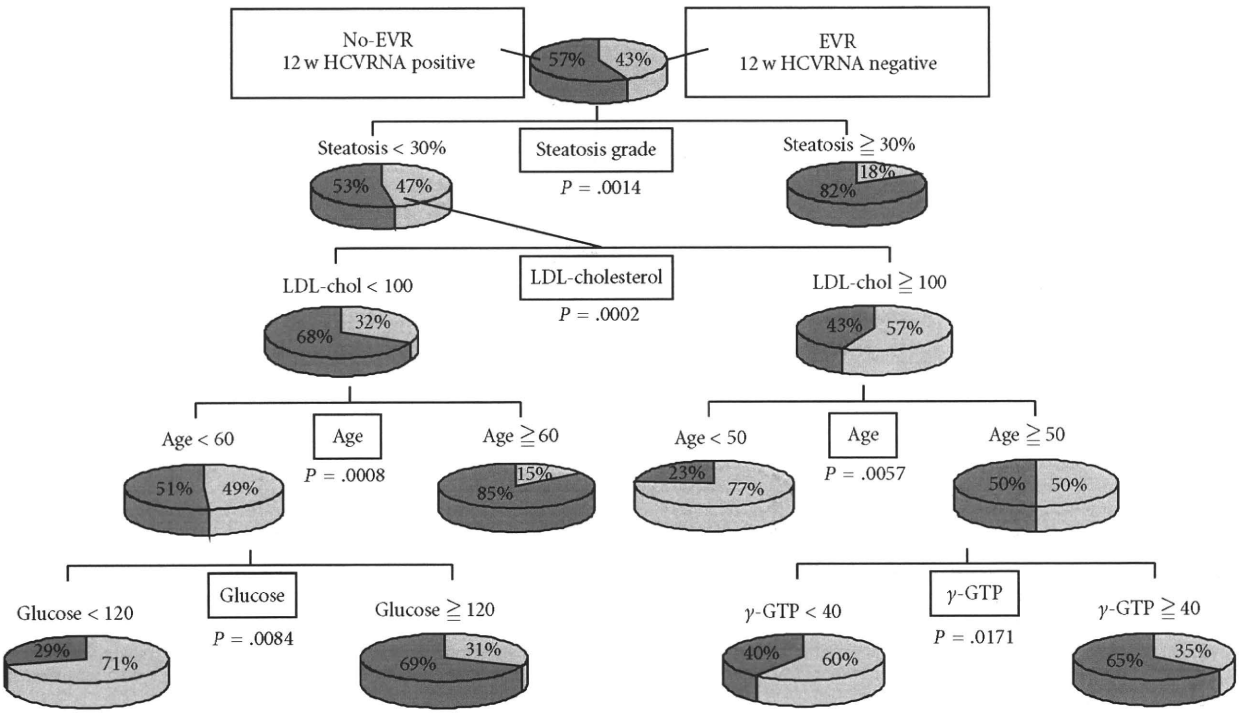

FIGURE 4: HCVRNA negative (EVR) algorithm at 12th week from data mining analysis of PEG-IFN alpha-2b plus RBV combination in the genotype 1b and high levels of HCVRNA. Both virological and host factors were evaluated by data mining analysis software from SPSS. This figure was cited from [36].

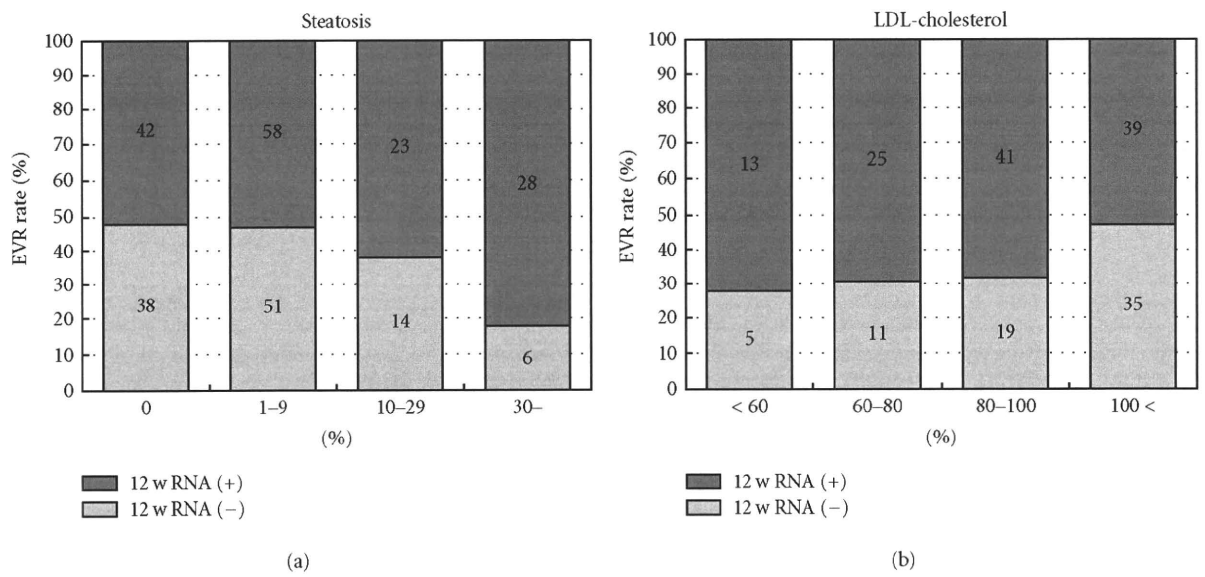

FIGURE 5: The rate of EVR in the patients with genotype 1b and high levels of HCVRNA, based on fatty deposition in the liver (a), and the LDL cholesterol value (b), respectively. EVR was highly achieved in the patients with less steatosis in the liver, and in those with high serum LDL-cholesterol levels. This is univariate analysis, and cited from [36].

We therefore compared these factors based on the original data. A univariate comparison of the fatty infiltration of the liver and the rate of EVR demonstrated that the rate of EVR was higher when the steatosis of the liver was less severe (Figure 5(a)). In addition, a comparison of the LDL cholesterol value and the rate of EVR demonstrated a significant correlation, confirming that the higher the LDL cholesterol value, the higher the rate of EVR (Figure 5(b)). Therefore, it was also proposed by the results of univariate analysis of each factor extracted from the data mining analysis that these factors were correlated to the rate of EVR.

From these observations, it is likely to improve the viral response to PEGIFN and ribavirin by reducing steatosis of the liver through daily walking or abstinating alcohol intake or by refraining from high-fat diet.

By utilizing data mining, it is therefore possible to assess virus- and host-related factors together and to predict the virological response in each patient, and thereby clinically useful information can be obtained. The algorithm should be validated including a large number of the patients.

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REVIEW

Recent advances of radiofrequency ablation for early hepatocellular carcinoma

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Key words
Hepatocellular carcinoma, radiofrequency ablation, seeding, transarterial chemoembolization, CLIP, BCLC, JIS, des-gamma-crboxy prothrombin time (DCP), alpha-fetoprotein (AFP).

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The author does not have any potentical conflicts of interest to disclose.

Abstract

Hepatocellular carcinoma (HCC) is the third leading cause of death in the malignant neoplastic diseases in the world. Surgical operation is sometimes not indicated because of complicated liver cirrhosis and extrahepatic disorders. Radiofrequency ablation has been developed as a less invasive treatment for HCC since 1999, and long-term outcome has been shown. There are several complications which should be paid attention, and to improve the prognosis, combination treatment with transarterial chemoembolization should be discussed. Overall survival after between RFA and surgical resection should be compared prospectively. Establishment of staging system for treatment allocation of HCC and prevention of HCC recurrence is important issue to be examined.

Introduction

Radiofrequency ablation (RFA) has been utilized as a less invasive and curative treatment for the treatment of hepatocellular carcinoma (HCC), and the methods and procedure have been developed. In some countries, it has been chosen as first line treatment for early stage HCC. Long-term prognosis has been reported and the associated factors for the prognosis after RFA have been shown. Several complications were reported after RFA. The prognosis was compared between patients who were treated by between surgical resection and those treated by RFA. The recent developments and future perspective of RFA is discussed in this review.

Radiofrequency ablation method

Of all therapeutic apparatus compared and evaluated up to now, the RF 3000 generator system (Boston Scientific, Boston, USA) had the most positive therapeutic effects.[1] However, in many articles, an internally cooled single electrode was used.[2] When there was a risk of RFA incurred by the hepatocellular carcinoma (HCC) location, the therapeutic effects were reduced, in particular the complete response rate was low in the vicinity of the gall bladder and the stomach and intestine, as well as the diaphragm, and in the vicinity of large blood vessels.[3] However, it has been reported that, although the therapeutic effects are not reduced when tumors exist in the vicinity of large blood vessels or adjacent to the extrahepatic organs, attention should be paid to the prevention and control of complications.[4] RFA with the use of artificial ascites for HCC adjacent to the diaphragm and to the stomach and intestine produced sufficient therapeutic effects, thereby improving the sonic window.[5]

When performing RFA, the use of a guiding needle with an external insulated sheath was useful because it allowed for precise tumor targeting.[6] The use of laparoscopic RFA has allowed a sufficient therapeutic effectiveness to achieve complete tumor ablation in all cases when the HCC nodule is located with bulging or at subcapsular area, as well as an adequate safety margin, compared to percutaneous RFA.[7] As shown in Fig. 1, extra-hepatic protruding HCC nodule is the most appropriate indication for laparoscopic RFA, and complete necrosis could be achieved after one treatment session under laparoscopic ultrasound guiding. When RFA was performed under laparoscopy, complete necrosis is usually observed.[8]

Assessment of the therapeutic effect of RFA

Although the effect of RFA is, in general, evaluated by dynamic computed tomography (CT) scans taken 1 to 7 days after the procedure, it was possible to assess the therapeutic effect by multidetector row helical CT (MD-CT) immediately after RFA,

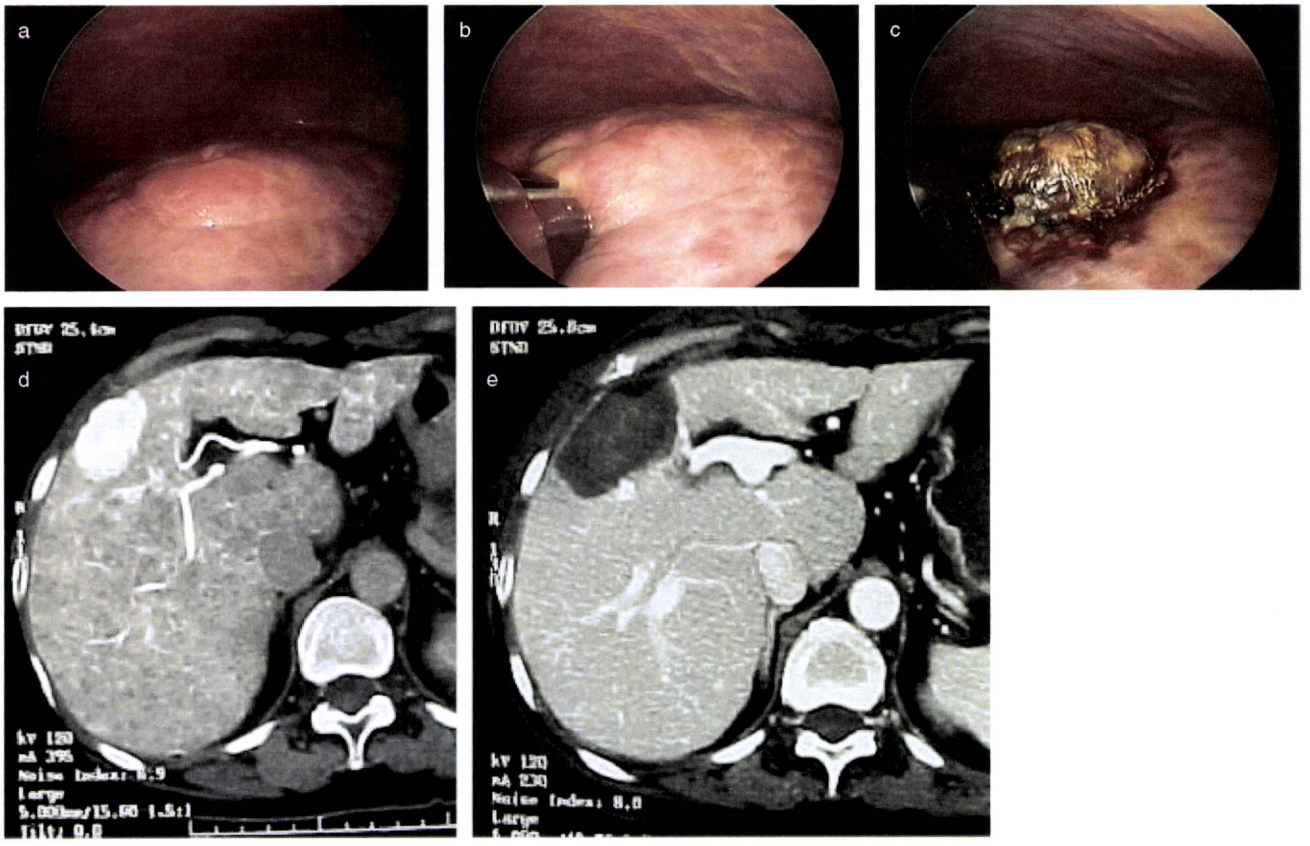

Figure 1 Hepatocellular carcinoma (HCC) nodule protruding from the liver surface is treated by laparoscopic radiofrequency ablation (RFA) under ultrasound guiding. (a) HCC nodule is directly observed under laparoscopy. (b) Under laparoscopic ultrasound guiding, RFA electrode is introduced to accurate position of the nodule, avoiding damage to diaphragm and intrahepatic vessels. (c) The entire HCC nodule was completely ablated by RFA. (d) Computed tomography (CT) scan before the treatment revealed hypervascular nodule with 2.6 cm in diameter at the surface of the liver. (e) After laparoscopic RFA, complete necrosis was confirmed by CT scan.

thereby achieving shorter hospital stays.[9] As well, one report indicates that it was possible to assess the therapeutic effect by contrast-enhanced ultrasonography immediately after RFA.[10] Contrast-enhanced sonography with abdominal virtual sonography was useful in monitoring the therapeutic effect and reducing the CT scan frequency.[11]

Prognosis after radiofrequency ablation

According to a report from a single institution in France, RFA was performed in 235 cases, with up to three HCC ≤ 5 cm in diameter, and achieved complete ablation in 222 cases. 67 cases were judged potentially resectable according to Barcelona Clinical Liver Cancer (BCLC) criteria; in these patients, RFA treatment produced 76% survival at 5 years. The factors contributing to survival were the prothrombin time and serum alpha-feto protein (AFP) levels. Conversely, the factors related to recurrence were multinodular tumors as well as the AFP level. In this report, RFA could be used as an effective first-line treatment in patients with a single nodule of 5 cm or less, a low serum AFP level, and well preserved liver function.[12]

According to a report from Italy, RFA was performed in 218 cases of single nodule HCC, measuring 2 cm or less in diameter, followed by an analysis of the prognosis. The 5-year survival rate was 68.5%, with a low 1.8% incidence of complications. Compared with resection, it was less invasive and could be conducted at a lower cost. It could therefore be considered the treatment of choice for resectable single HCC ≤ 2.0 cm.[13] In Japan, the prognosis of 1000 patients who had undergone RFA was analyzed; the 1, 3, and 5-year survival rates were 94.7%, 77.7% and 54.3%, respectively.[2] According to a report from China, the factors related to the prognosis after RFA were the tumor diameter, the number of tumors, the use of combination therapy with ethanol injection, the margin, and the Child-Pugh score.[14] According to the outcome of RFA treatment for a large single-institution series in Korea, the method had a local recurrence rate of 8.1% at 1 year and 11.8% at 3 years, and patient survival rates were 95.2% at 1 year, 69.5% at 3 years, and 58.0% at 5 years.[15] The five year survival after RFA was similar between Western and Eastern countries (Table 1).

Prognosis after RFA and the staging system

The Cancer of the Liver Italian Program (CLIP) score and BCLC scoring system more accurately predicted the prognosis than the Okuda score in patients with early-intermediate HCC, undergoing

Table 1 5-year overall survival after radiofrequency ablation in the patients with operable HCC nodule

		Overall survival		
Investigator	Diameter of the nodule	Patient number	3 y	5y
N'Kontchou G[16]	≤ 5 cm	235		76%
Livraghi T[17]	≤ 2 cm	218		68.5%
Tateichi R[18]	≤ 3 cm	1000	77.7%	54.3%
Peng ZW[19]	≤ 5 cm	281	57.1%	37.1%
	≤ 3 cm		65.7%	58.6%
Choi D[20]		570	69.5%	58.0%

non-surgical therapy, such as RFA.[21] The results of an analysis in Japan demonstrated that, regardless of the CLIP score, the combination of transarterial chemoembolization (TACE)—RFA had the highest 5-year survival.[22] In Japan, where early-stage HCC is prevalent, the majority of cases are classified into CLIP stage 1 of CLIP scores and, as such, the Japanese integrated staging (JIS) score was proposed as a new early HCC staging system.[23] The results of the validation done in many cases demonstrated that the JIS score yielded a better prediction of the prognosis than the CLIP score.[24] It has also been reported from in Korea that the JIS score is the most appropriate score for predicting the prognosis.[25]

Tumor markers

The tumor marker relevant to the prognosis after RFA is des-gamma-carboxy prothrombin time (DCP) levels; wherein, high DCP levels predicted a poor prognosis after RFA.[16,17] However, the same institution also reported that a comparison of AFP, DCP and AFP-leptin 3 (AFP-L3) demonstrated that AFP-L3 was the most useful indicator of the overall survival and disease-free survival.[18] It was pointed out that the AFP mRNA levels in the blood after RFA are also an objective index of recurrence.[19] On the other hand, blood vascular endothelial growth factor (VEGF) levels have also been reported to be related to the prognosis.[20]

Recurrence

Local tumor recurrence after RFA is 9.0% at 1 year and 17.7% at 3 years; therefore, local recurrence is relevant to the prognosis for survival.[26] Evaluation of the therapeutic effects of RFA by contrast enhanced CT scans or by enhanced magnetic resonance imaging (MRI) here demonstrated that the procedure provides good local control and the recurrence rate is low in cases in which the post-ablation margin was 0.4 cm or more and the tumor size was smaller than 2.5 cm.[27] The overall local recurrence rate after RFA was 12.8% and the tumor diameter of >2.5 cm was a significant independent factor.[28] However, another report indicates that even when local recurrence occurred, it did not adversely affect the survival prognosis.[29] Utilizing the RF 3000 generator system has been reported more positive effects than cool-tip electrode.[30]

On the other hand, the cumulative rate of intrahepatic distant recurrence was reported as 10.4% and 77.0% at 1 and 5 years, respectively. In a multivariate analysis, AFP and DCP values, as well as the safety margin, were significant independent factors.[31] The intra-hepatic distant recurrence was associated with multi-nodular lesions and hepatitis C virus (HCV), even after curative ablation was achieved.[32] Recurrence at a distant site is an important, poor prognostic factor.[33] Although it is possible to ensure long-term survival by carrying out repeat RFA after recurrence,[34] the more frequently recurrences occur, the higher the risk for subsequent recurrence becomes.[35] Histological grade is relevant to the therapeutic efficiency of RFA and also plays a part in determining survival.[36]

Prognosis and possible measures to improve survival after RFA

Long-term interferon maintenance therapy improved the survival in patients with HCV related HCC after RFA.[37] On the other hand, the administration of lamivudine after RFA for hepatitis B virus (HBV)-related HCC maintained the liver function and was also safe.[38,39] The administration of vitamin K for HCV-related HCC did not produce a chmenopreventative effect.[40] The oral administration of a branched-chain amino acid after RFA made it possible to maintain the serum albumin levels and it was also useful for improving the liver function.[41]

Resection *versus* RFA

With regard to the question of whether surgical resection or RFA is superior, two randomized comparisons have been reported—all from China. In these reports, the life prognoses of single HCCs of 2 cm or less diameter were randomly compared between RFA and resection. It was reported that there would be no difference between the two, or that, for single HCC of 5 cm or less, there was no difference in terms of both disease-free survival and overall survival.[42,43] In Italy, a group of 109 patients who underwent RFA and a group of 91 patients who underwent resection were compared retrospectively; there was no difference in terms of the overall survival and disease-free survival, for HCC of 3 cm or less.[44] Likewise, a retrospective analysis conducted in Korea, compared a group of 55 patients who underwent RFA treatment for single HCC 4 cm or less and well-preserved liver function with a group of 93 patients who underwent resection; the authors concluded that there was no difference in terms of overall survival and recurrence-free survival at 1 year and 3 years after RFA.[45] When laparoscopic RFA was performed on patients with single HCC nodule with Child-Pugh A liver function, RFA and resection had similar survival rates.[46,47]

However, a case control study of resection versus RFA showed that recurrence, tumor diameter, and whether resection or RFA were performed, all affected overall survival. The authors con-