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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Pretreatment prediction of response to peginterferon plus ribavirin therapy in genotype 1 chronic hepatitis C using data mining analysis

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Abstract
Background This study aimed to develop a model for the pre-treatment prediction of sustained virological response (SVR) to peg-interferon plus ribavirin therapy in chronic hepatitis C.
Methods Data from 800 genotype 1b chronic hepatitis C patients with high viral load (>100,000 IU/ml) treated by peg-interferon plus ribavirin at 6 hospitals in Japan were randomly assigned to a model building ($n = 506$) or an internal validation ($n = 294$). Data from 524 patients treated at 29 hospitals in Japan were used for an external validation. Factors predictive of SVR were explored using data mining analysis.
Results Age (<50 years), alpha-fetoprotein (AFP) (<8 ng/mL), platelet count ($\geq 120 \times 10^9$/l), gamma-glutamyl-transferase (GGT) (<40 IU/l), and male gender were used to build the decision tree model, which divided patients into 7 subgroups with variable rates of SVR ranging from 22 to 77%. The reproducibility of the model was confirmed by the internal and external validation ($r^2 = 0.92$ and 0.93, respectively). When reconstructed into 3 groups, the rate of SVR was 75% for the high probability group, 44% for the intermediate probability group and 23% for the low probability group. Poor adherence to drugs lowered the rate of SVR in the low probability group, but not in the high probability group.

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Conclusions A decision tree model that includes age, gender, AFP, platelet counts, and GGT is useful for predicting the probability of response to therapy with peg-interferon plus ribavirin and has the potential to support clinical decisions regarding the selection of patients for therapy.

Keywords Data mining · Decision tree · Alpha-fetoprotein · HCV · Peg-interferon

Introduction

The current standard therapy for genotype 1 chronic hepatitis C is 48 weeks of pegylated interferon (PEG-IFN) plus ribavirin (RBV) [1]. Sustained virological response (SVR), defined as undetectable HCVRNA post-treatment is regarded as a cure of chronic hepatitis C. However, the rate of SVR to this regimen is only 50% in patients with HCV genotype 1b and a high HCVRNA titer [2, 3]. Since PEG-IFN and RBV combination therapy is costly and accompanied by potential adverse effects, the ability to predict the possibility of SVR before therapy may significantly influence the selection of patients for therapy. A recent report revealed that single nucleotide polymorphisms located in the *IL28B* are strongly associated with a response to PEG-IFN plus RBV therapy [4–6]. Besides, the amino acid substitutions in the NS5A [7–9] or core region of HCV were also associated with response to therapy [10, 11]. Unfortunately, these host genetic and viral factors are not yet readily available for general application in actual clinical practice. Fibrosis of the liver is also an important predictor of response, but resources may be limited in some countries. Clinical and non-invasive parameters may be better suited for general practice, but there is no established means by which the likelihood of a response can be predicted prior to therapy.

Data mining is a method of predictive analysis that explores data, without setting the hypothesis, to discover hidden patterns and relationships in highly complex datasets and enables the development of predictive models. Decision tree analysis is a core component of data mining and predictive modeling [12], and it is utilized by decision makers in various fields of business. Recent publications on decision tree analysis indicate its usefulness for defining prognostic factors in various diseases such as prostate cancer [13], diabetes [14], melanoma [15, 16], colorectal carcinoma [17, 18], and liver failure [19]. The results of the analysis are presented as a tree structure, which is intuitive and facilitates the allocation of patients into subgroups by following the flow chart form [20]. We have recently reported the usefulness of decision tree analysis for the prediction of early virological response (undetectable HCVRNA within 12 weeks of therapy) to PEG-IFN and RBV combination therapy in chronic hepatitis C [21].

In the present study, we used decision tree analysis to explore baseline predictors of response to PEG-IFN/RBV therapy so that a pre-treatment algorithm could be created to discriminate chronic hepatitis C patients who are likely to respond to PEG-IFN/RBV therapy from those who are not. For the purpose of use in general practice, only clinical and non-invasive parameters were included in the analysis.

Materials and methods

Patients

This was a multicenter retrospective cohort study supported by the Japanese Ministry of Health, Labor and Welfare. Data were collected from a total of 800 chronic hepatitis C patients who received therapy for 48 weeks with PEG-IFN alpha-2b and RBV at Musashino Red Cross Hospital, Toranomon Hospital, Tokyo Medical and Dental University, Osaka University, Nagoya City University Graduate School of Medical Sciences, Yamanashi University, and their related hospitals. The inclusion criteria to be enrolled in this study were as follows (1) infection by genotype 1b, (2) HCVRNA higher than 100,000 IU/ml by quantitative PCR (Cobas Amplicor HCV Monitor v 2.0, Roche Diagnostic systems, CA), which is typically used for the definition of high viral load in Japan, (3) lack of co-infection with hepatitis B virus or human immunodeficiency virus, (4) lack of other causes of liver disease such as autoimmune hepatitis and primary biliary cirrhosis and (5) completion of at least 12 weeks of therapy. Patients received PEG-IFN alpha-2b (1.5 μg/kg) subcutaneously every week and were administered a weight-adjusted dose of RBV (600 mg for <60 kg, 800 mg for 60–80 kg, and 1,000 mg for >80 kg), which is the recommended dosage in Japan. Patients who were treated for more than 49 weeks were not included in the study. For the analysis, patients were randomly assigned to either the model building ($n = 506$) or the internal validation ($n = 295$) group. Consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional review committee. The baseline characteristics and representative laboratory test results are listed in Table 1. The overall rate of SVR was 47% in the model building set and 49% in the validation set. There were no significant differences in the clinical backgrounds between these 2 groups.

For external validation of the model, we collaborated with another study group supported by the Japanese Ministry of Health, Labor and Welfare. This multicenter study group consisted of 29 medical centers and hospitals

Table 1 Comparison of pre-treatment factors between model building and internal validation patients

	Model ($n = 506$)	Validation ($n = 295$)
Age (years)	56 (14–75)	55 (18–74)
Male gender[a]	261/506 (52%)	160/295 (54%)
Body mass index (kg/m^2)	22.9 (14.3–34.0)	23.2 (16.1–33.8)
Albumin (g/dl)	4 (2.7–5.0)	4 (2.8–4.9)
Creatinine (mg/dl)	0.7 (0.4–1.5)	0.7 (0.4–1.1)
AST (IU/l)	60 (11–370)	62 (11–240)
ALT (IU/l)	73 (11–413)	73 (14–390)
GGT (IU/l)	56 (10–328)	55 (7–409)
Total cholesterol (mg/dl)	173 (73–297)	171 (29–273)
Triglyceride (mg/dl)	105 (33–474)	109 (32–372)
White blood cell count (/μl)	4,745 (1,800–10,900)	4,823 (1,200–9,700)
Neutrophil count (/μl)	2,563 (667–7,870)	2,484 (508–7,579)
Red blood cell count (/μl)	448 (313–577)	451 (313–574)
Hemoglobin (g/dl)	14.1 (9.4–18.3)	14.1 (10.0–18.0)
Hematocrit (%)	41.7 (13.3–53.7)	41.9 (15.5–52.7)
Platelets (10^9/l)	164 (52–380)	158 (43–312)
AFP (ng/ml)	14.7 (0.9–680)	13 (0.8–323)
HCVRNA (10^3 IU/ml)	1,852 (100–5,100)	1,870 (100–5,100)
Fibrosis stage: F3–4	73/417 (18%)	48/247 (19%)

Data expressed as median (range) unless otherwise indicated

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyltransferase, *AFP* alpha-fetoprotein

[a] Data expressed as number/available data (percentage)

belonging to the National Hospital Organization. A dataset collected from 524 patients who were treated with PEG-IFN alpha-2b/RBV was used as an external validation dataset, i.e., completely independent from the dataset that was used for model building.

Laboratory tests

Blood samples were obtained before therapy and at least once every month during therapy, and were used for hematologic tests, blood chemistry analysis and determination of HCV RNA. Pretreatment levels of HCV RNA were quantified by Cobas Amplicor (Roche Diagnostic Systems, Pleasanton, CA). SVR was defined as undetectable HCV RNA at week 24 after completion of therapy, as determined by qualitative PCR with a lower end detection limit of 50 IU/ml (Amplicor, Roche Diagnostic Systems). Liver biopsy was available in 664 patients. Fibrosis and activity were scored according to the METAVIR scoring system [22]. Fibrosis was staged on a scale of 0–4: F0 (no fibrosis), F1 (mild fibrosis: portal fibrosis without septa), F2 (moderate fibrosis: few septa), F3 (severe fibrosis: numerous septa without cirrhosis) and F4 (cirrhosis). Activity of necroinflammation was graded on a scale of 0–3: A0 (no activity), A1 (mild activity), A2 (moderate activity) and A3 (severe activity).

Statistical analysis

A database of pretreatment variables was created containing 6 variables from hematological tests (red blood cells, hemoglobin, hematocrit, white blood cells, neutrocytes and platelets), 8 variables from the blood chemistry test [creatinine, albumin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase (GGT), total cholesterol, triglyceride and alpha-fetoprotein (AFP)], serum level of HCV RNA and 3 variables for patient characteristics (age, gender and body mass index). Based on this database, the recursive partitioning analysis algorithm referred to as decision tree analysis was implemented to define meaningful subgroups of patients with respect to the possibility of achieving SVR.

Decision tree analysis is a family of nonparametric regression methods. Software is used to automatically explore the data to search for optimal split variables and to build a decision tree structure [23]. For the analysis, the entire study population was evaluated to determine which variables and cutoff points yielded the most significant division into 2 prognostic subgroups that were as homogeneous as possible for the probability of SVR. Thereafter, the same analytic process was applied to all newly defined subgroups. A restriction was imposed on the tree construction such that the procedure stopped when either no additional significant variable was detected or when the sample size was below 20. For this analysis, the data mining software IBM SPSS Modeler 13 (IBM SPSS Inc., Chicago, IL) was utilized. SPSS software v.15.0 (SPSS Inc., Chicago, IL) was used for multivariate logistic regression analysis.

Results

Decision tree analysis

Decision tree analysis was carried out on the model building dataset from 506 patients using 18 variables. Figure 1 shows the results. The analysis automatically selected 5 predictive variables to produce a total of 7 subgroups of patients. Age was selected as the variable of initial split with an optimal cutoff of 50 years. The possibility of achieving SVR was 41% for patients older than 50 compared to 70% for patients

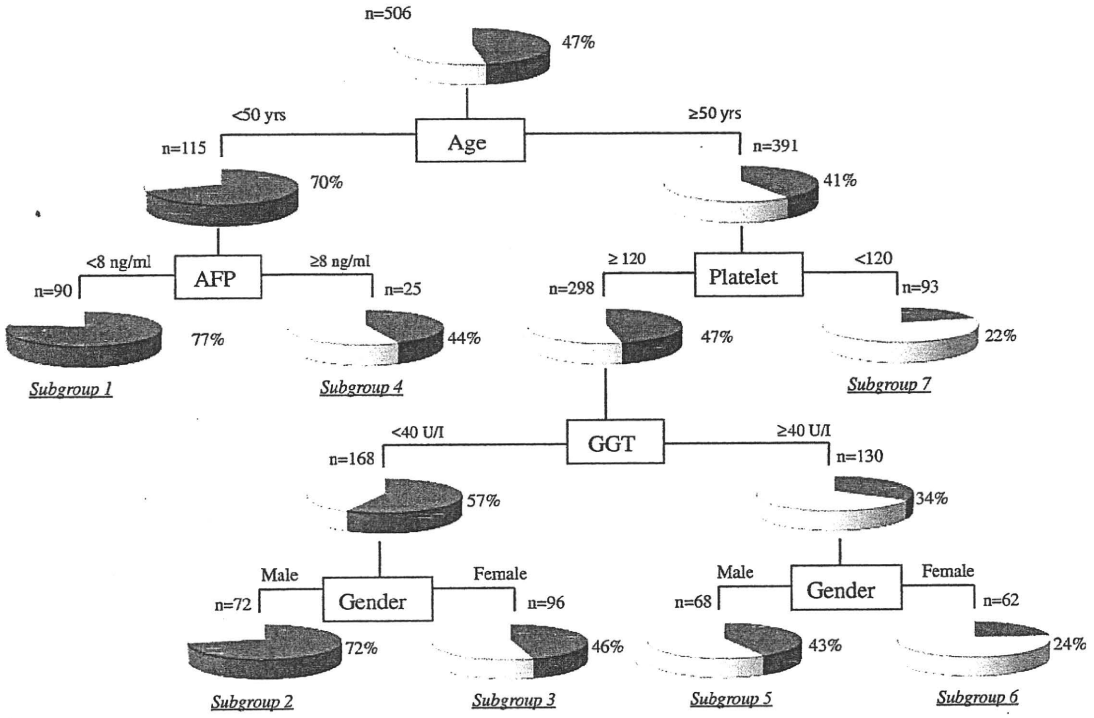

Fig. 1 Decision tree analysis. *Boxes* indicate the factors for splitting and the cutoff value for the split. *Pie charts* indicate the rate of SVR for each group. Terminal subgroups of patients discriminated by the analysis are numbered from 1 to 7. *AFP* alpha-fetoprotein, *GGT* gamma-glutamyltransferase

younger than 50. Among patients younger than 50, the level of serum AFP, with an optimal cutoff of 8 ng/ml, was selected as the variable of second split. Patients with lower AFP levels had a higher probability of SVR (77 vs. 44%). Among older patients, platelet count was selected as the second variable of split, with an optimal cutoff of 120×10^9/l. Patients with higher platelet counts had a higher probability of SVR (47 vs. 22%). Among patients with platelet counts higher than 120×10^9/l, GGT was selected as the third variable of split with an optimal cutoff of 40 IU/l. Patients with a lower GGT level had a higher probability of SVR (57 vs. 34%). Gender was selected as the fourth variable of split, with male gender being a predictor of a higher SVR probability (72 vs. 46% in patients with GGT levels <40 IU/l and 43 vs. 24% in those with GGT ≥40 IU/l). HCVRNA load was included in the analysis but was not selected as a significant variable.

The probabilities of SVR for the 7 subgroups derived by this process were highly variable. The subgroup of young patients (<50 years) with low serum AFP (<8 ng/ml) (subgroup 1) or the subgroup of older (≥50 years) male patients with high platelet counts ($\geq 120 \times 10^9$/l) and low serum GGT (<40 IU/l) (subgroup 2) showed the highest probability of SVR (72 and 77%), while the subgroup of older (≥50 years) patients with low platelet counts ($<120 \times 10^9$/l) (subgroup 7) and older (≥50 years) female patients with high serum GGT (subgroup 6) showed the lowest probability of SVR (22 and 24%).

Validation of the decision tree

The results of the decision tree analysis were validated with an internal validation dataset of 295 cases, which was independent of the model building dataset. Each patient in the validation set was allocated to subgroups 1–7 using the flow-chart form of the decision tree. The rates of SVR were 77% for subgroup 1, 71% for subgroup 2, 55% for subgroup 3, 44% for subgroup 4, 41% for subgroup 5, 17% for subgroup 6, and 30% for subgroup 7. The rates of SVR for each subgroup of patients were closely correlated between the model building dataset and the internal validation dataset ($r^2 = 0.925$) (Fig. 2a).

To further confirm the universality of the results, data collected from 524 patients by a collaborating study group were used for external validation. Thus, the dataset used for external validation was completely independent of the

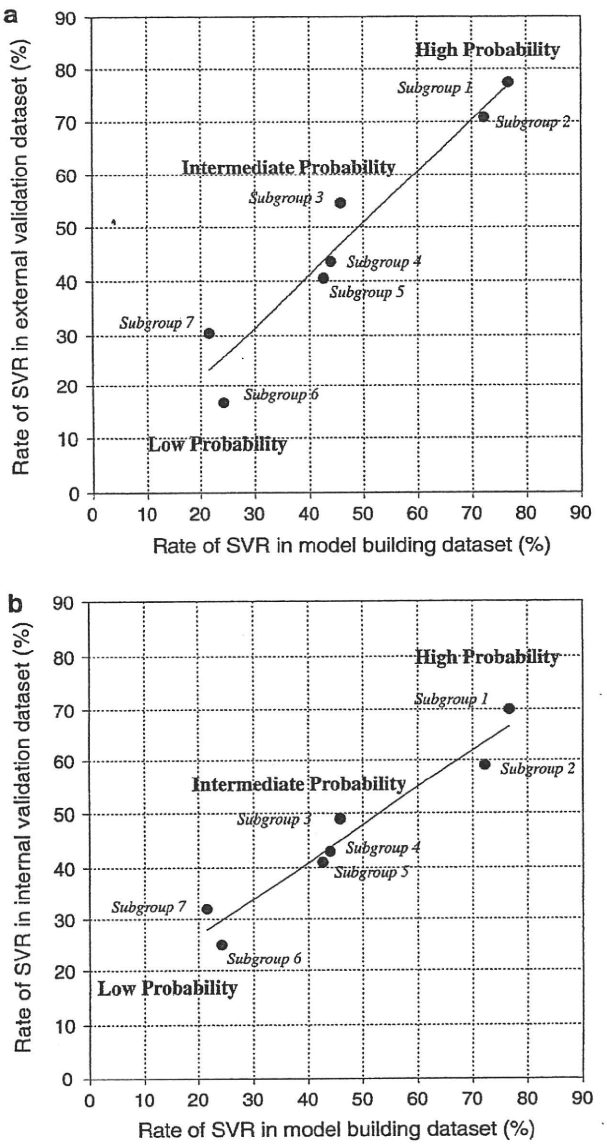

Fig. 2 Validation of the decision tree analysis by an internal and external validation dataset: subgroup-stratified comparison of the SVR rate. The rate of SVR in each subgroup was plotted. The *X* axis represents the model building, and the *Y* axis represents the validation datasets. **a** Internal validation and **b** external validation. There was a close correlation between the model building and the internal validation dataset (correlation coefficient $r^2 = 0.925$) and between the model building and the external validation dataset (correlation coefficient $r^2 = 0.936$)

original dataset used for model building. Each patient in the external validation set was allocated to subgroups 1–7 using the flow-chart form of the tree. The rates of SVR were 70% for subgroup 1, 59% for subgroup 2, 49% for subgroup 3, 43% for subgroup 4, 41% for subgroup 5, 25% for subgroup 6, and 32% for subgroup 7. The rates of SVR for each subgroup of patients were closely correlated

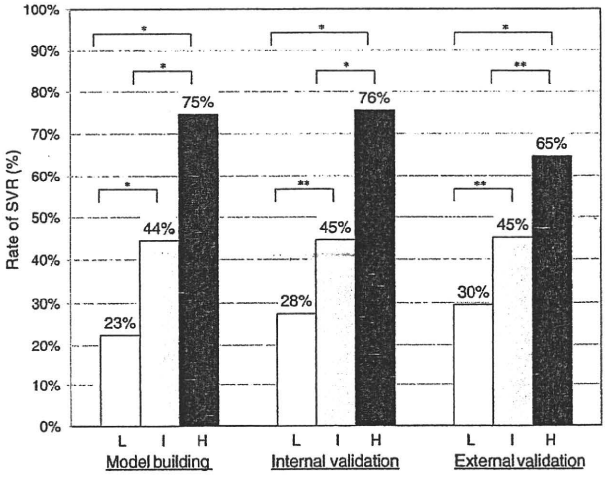

Fig. 3 Comparison of SVR rates between groups divided by the decision tree. The rate of SVR was compared among the 3 groups of patients divided by the decision tree analysis (*white*, *gray* and *black boxes*, indicating a low (*L*), intermediate (*I*) and high (*H*) probability group, respectively). The rate of SVR was significantly different among the 3 groups. $*p < 0.0001$, $**p < 0.001$

between the model-building dataset and the validation dataset ($r^2 = 0.936$) (Fig. 2b).

Construction of 3 groups according to the probability of SVR

Seven subgroups were reconstructed into 3 groups according to their predicted rates of SVR: the high probability group consisted of subgroups 1 and 2, the intermediate probability group consisted of subgroups 3, 4 and 5, and the low probability group consisted of subgroups 6 and 7. The rate of SVR was significantly different among the 3 groups (Fig. 3). The rate of SVR in the high probability group was consistently high: 75% for model building patients, 76% for internal validation patients and 65% for external validation patients. Conversely, the rate of SVR in the low probability group was consistently low: 23% for model building patients, 28% for internal validation patients and 30% for external validation patients. The rate of SVR in the intermediate probability group was 44% for model building patients, 45% for internal validation patients and 45% for external validation patients. Since 28–32% of patients were classified as high probability and 30–32% were classified as low probability, roughly 60% of patients were classified as having either a high or low probability of achieving SVR.

Effect of dose reductions of PEG-IFN and RBV on SVR

The cumulative dose of PEG-IFN and RBV was not included as a variable of analysis since the present study

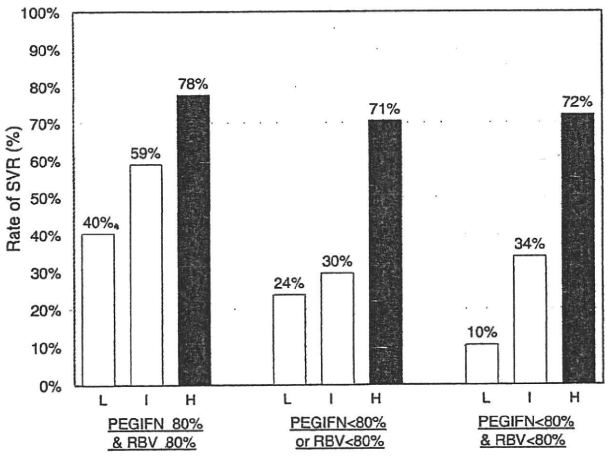

Fig. 4 Comparison of SVR rates among groups stratified by drug adherence. The 3 groups of patients divided by the decision tree analysis (*white*, *gray* and *black boxes* indicating a low (*L*), intermediate (*I*) and high (*H*) probability group, respectively) were further stratified according to the cumulative drug exposure of PEG-IFN and RBV. The good adherence group (≥80% planned dose of both PEG-IFN and RBV) had a higher rate of SVR compared with the poor adherence group (<80% planned dose of both PEG-IFN and RBV) in the low ($p = 0.0003$) and intermediate ($p = 0.007$) but not in the high probability group ($p = 0.53$)

aimed to develop a pre-treatment model for the prediction of response. To analyze the possible effect of drug reductions on the result of the decision tree analysis, 3 groups of patients divided by the decision tree analysis (low, intermediate and high probability group) were further stratified according to the cumulative drug exposure of PEG-IFN and RBV (Fig. 4). Even after adjustment for adherence, 3 groups of patients still had low, intermediate and high probability of achieving SVR, respectively. Of note, the good adherence group (≥80% planned dose of both PEG-IFN and RBV) had higher rates of SVR compared with the poor adherence group (<80% planned dose of both PEG-IFN and RBV) in the low ($p = 0.0003$) and intermediate ($p = 0.007$) probability group, but not in the high probability group ($p = 0.53$).

Factors associated with SVR by multivariate logistic regression analysis

We also explored the factors associated with SVR using a standard statistical analysis. By univariate analysis, age, gender, serum albumin, creatinine, alanine aminotransferase, GGT, red blood cell count, hemoglobin, hematocrit, platelet count and AFP were found to be associated with SVR (Table 2). HCVRNA load was not associated with SVR. By multivariate analysis, age, gender, GGT and platelet count were found to be independently associated with SVR (Table 3). Of note, AFP, which was selected as a significant predictor of response in the decision tree analysis, was not found to be an independent response predictor in the standard multivariate analysis. This indicates a unique feature of the decision tree analysis; i.e., it could identify significant predictors that specifically apply to selected patients, in this case patients younger than 50 years old.

Relationships between decision tree model and stage of fibrosis or HCV RNA load

Liver biopsy was performed in 664 patients. The distribution of fibrosis in three probability groups differed significantly. Advanced fibrosis (F3 or F4) was higher in the low probability group (39%) compared to the intermediate probability group (13%) ($p < 0.0001$) and to the high probability group (6%) ($p < 0.0001$). Advanced fibrosis was also higher in the intermediate group compared to the high probability group ($p = 0.01$). AFP was significantly associated with liver fibrosis stage: medians of AFP levels were 4.9, 5.9, 13.0 and 18.6 for F1, F2, F3 and F4, respectively ($p < 0.0001$, Spearman's rank correlations). Lower platelet counts correlated with advanced fibrosis stages (data not shown). The SVR rate was higher in the high probability group compared to the intermediate or low probability group after stratification by HCV RNA load. Among patients with low HCVRNA load (<400,000 IU/ml), the rate of SVR was 93, 59 and 50% for the high, intermediate and low probability group, respectively ($p = 0.002$ for high vs. intermediate and $p < 0.001$ for high vs. low probability groups). Among patients with a high HCVRNA load (≥400,000 IU/ml), the rate of SVR was 73, 42 and 21% for the high, intermediate and low probability group, respectively ($p < 0.001$ for high vs. low, high vs. intermediate and intermediate vs. low probability groups).

Discussion

Currently, the combination of PEG-IFN and RBV is the recommended therapy for chronic HCV infection. The rate of SVR with 48 weeks of therapy is around 50% in patients with HCV genotype 1b and a high HCV RNA titer [2, 3]. To date, the virological response during therapy is the most reliable means for predicting the likelihood of SVR [2, 24, 25]. More potent therapy, such as a triple combination of protease inhibitor, PEG-IFN and RBV, is being evaluated in clinical trials but is not readily available [26, 27]. Under the circumstances, pre-treatment prediction of the likelihood of SVR may be useful for both patients and physicians to support clinical decisions as to whether to start PEG-IFN/RBV therapy or delay treatment until a new more effective therapy becomes available.

Table 2 Comparison of pre-treatment factors between patients with and without sustained virological response (SVR) among the model building dataset ($n = 506$)

	SVR ($n = 240$)	Non-SVR ($n = 266$)	p
Age (years)	54 (25–75)	60 (36–73)	<0.0001
Male gender[a]	151/240 (63%)	171/266 (41%)	<0.0001
Body mass index (kg/m^2)	22.5 (16.8–32.0)	22.6 (15.5–33.3)	0.244
Albumin (g/dl)	4.1 (3.2–5.0)	4 (2.7–4.9)	0.004
Creatinine (mg/dl)	0.7 (0.44–1.14)	0.69 (0.39–1.47)	<0.0001
AST (IU/l)	59 (11–370)	61 (17–261)	0.457
ALT (IU/l)	58 (11–413)	53 (11–316)	0.031
GGT (IU/l)	31 (10–322)	43 (12–328)	0.005
Total cholesterol (mg/dl)	175 (87–297)	171 (73–274)	0.184
Triglyceride (mg/dl)	105 (36–474)	105 (33–294)	0.992
White blood cell count (/μl)	4,600 (2,200–10,900)	4,425 (1,800–10,810)	0.479
Neutrophils (/μl)	2,507 (667–7,870)	2,423 (900–7,281)	0.321
Red blood cell count (/μl)	455 (336–577)	441 (313–564)	0.001
Hemoglobin (g/dl)	14.3 (10.2–17.6)	13.9 (9.4–17.9)	0.004
Hematocrit (%)	42.1 (13.3–53.7)	41.2 (30.7–52.0)	0.031
Platelets (10^9/l)	178 (81–380)	142 (60–320)	<0.0001
AFP (ng/ml)	4.3 (0.9–680)	6.4 (1.9–468)	0.041
HCVRNA (10^3 IU/ml)	1,400 (100–5,100)	1,700 (100–5,100)	0.659
Fibrosis stage: F3–4[a]	21/198 (11%)	52/219 (24%)	<0.0001

Data expressed as median (range) unless otherwise indicated

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyltransferase, *AFP* alpha-fetoprotein

[a] Data expressed as number/available data (percentage)

Table 3 Multivariate logistic regression analysis for factors associated with sustained virological response (SVR)

	Odds	95% CI	p value
Age (years)	0.96	0.94–0.98	0.001
Platelets (10^9/l)	1.09	1.04–1.14	<0.0001
ALT (IU/l)	1.01	1.00–1.01	0.001
GGT (IU/l)	0.99	0.98–0.99	<0.0001
Male gender	2.92	1.87–4.55	<0.0001

GGT gamma-glutamyltransferase

Using the data mining analysis, we constructed a simple decision tree model for the pre-treatment prediction of response to PEG-IFN/RBV. The analysis highlighted 5 variables relevant to response: age, gender, platelet count, AFP and GGT. Classification based on these variables identified subgroups of patients with high probabilities of achieving SVR among difficult to treat genotype 1b chronic hepatitis C patients. The reproducibility of the model was confirmed by the independent internal and external validation datasets. An advantage of the decision tree analysis over traditional regression models is that the decision tree model is user-intuitive and can be readily interpreted by medical professionals without any specific knowledge of statistics. Patients can be allocated to specific subgroups with a defined rate of response simply by following the flow-chart form. Using this model, an estimate of the response before treatment can be rapidly obtained, which may facilitate clinical decision making. Thus, this model could be readily applicable to clinical practice.

According to the results of the decision tree analysis, patients were categorized into 3 groups: the rate of SVR was 23–30% for the low probability group, 44–45% for the intermediate probability group and 65–76% for the high probability group. About 30% of patients were each categorized in the high and low probability group and the remaining 40% of patients in the intermediate probability group. These results support the evidence-based approach for selecting an optimum treatment strategy for individual patients. For example, patients in the high probability group may be the most suitable candidates for PEG-IFN/RBV therapy, while patients in the low probability group may be advised to wait for a future therapy, such as the combination of protease inhibitor, PEG-IFN and RBV. However, the estimation of low probability should not be used to preclude patients from therapy, and the final decision should be made on a case-by-case basis, taking into consideration the acceptance by the patient of a low likelihood of response and the potential risk of disease progression while waiting for a future therapy.

Another important finding was that poor adherence to drugs lowered the rate of SVR in the low and intermediate probability groups, which implies that effort should be made to maintain ≥80% of the planned dose of PEG-IFN and RBV in those patients. On the other hand, the rate of SVR was high irrespective of drug adherence in the high probability group. Whether shorter duration of therapy is sufficient in this group of patients should be confirmed in future study.

The variables used in the decision tree have been previously reported to associate with the efficacy of IFN therapy. Younger age and male gender are associated with a favorable response [28]. Lower platelet count is a hallmark of advanced fibrosis in chronic hepatitis C and is reported to be associated with poor response to IFN [29]. AFP is usually used for the screening or the diagnosis of hepatocellular carcinoma, but recent studies suggest an association between higher AFP levels and poor response to IFN therapy [30–33]. Previous report speculated that higher expression of AFP by hepatic progenitor cells may be associated with non-response to therapy [30]. Another report speculated that AFP levels predict poor response to therapy through the underlining link to advanced liver fibrosis [31]. Our data support the latter speculation since advanced fibrosis was associated with elevation of AFP levels. Fibrosis of the liver is an important predictor of response, but we did not include this factor in the decision tree analysis since liver biopsy may not always be available in general practice. As a result, two predictive factors that correlate with fibrosis stage (platelet counts and AFP) were selected in the model, and three probability groups reflected the different distribution of fibrosis stage. GGT is reported to be associated with insulin resistance and hepatic steatosis [34–37], a factor that confers resistance to IFN therapy [38–44]. What is unique to the present study is the visualization of response probability by combining these factors and its high reproducibility revealed by a high-quality validation of the model by internal and external validation datasets that were completely independent of the model building dataset. Since factors used in the model were clinical parameters that are readily available by the usual workup of patients, this model could be immediately applicable to clinical practice without imposing costs for additional examinations.

A potential limitation of this study is that data mining analysis has an intrinsic risk of showing relationships that fit to the original dataset but are not reproducible in different populations. Although internal and external validations showed that our model had high reproducibility, we recognize that further validation on a larger external validation cohort, especially in populations other than Japanese, may be necessary to further verify the reliability of our model.

In conclusion, we built a pre-treatment model for the prediction of virological response to PEG-IFN/RBV. Because this decision tree model was made up of simple variables, it can be easily applied to clinical practice. This model may have the potential to support decisions about patient selection for PEG-IFN/RBV based on a possibility of response weighed against the potential risk of adverse events or costs.

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ORIGINAL ARTICLE

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

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Abstract

Purpose Lamivudine treatment of chronic hepatitis B (CHB) is associated with frequent resistance and loss of clinical benefit. We present outcomes of lamivudine-refractory Japanese patients treated with entecavir for 3 years.

Methods Eighty-two patients refractory to lamivudine therapy received entecavir 0.5 or 1 mg daily for 52 weeks in phase II study ETV-052, directly entered rollover study ETV-060, and received entecavir 1 mg daily. Responses were evaluated among patients with available samples.

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. Sixteen patients in the 1-mg subset had baseline and week 148 evaluable biopsy pairs: 81% (13/16) showed histologic improvement and 38% (6/16) showed

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improvement in fibrosis. Genotypic resistance to entecavir emerged in 31 patients for a 3-year cumulative resistance probability of 35.9%. Entecavir was generally well tolerated during ETV-060, with no on-treatment ALT flares.
Conclusions Long-term entecavir treatment of lamivudine-refractory CHB resulted in virologic suppression, ALT normalization, and improvements in liver histology. Resistance was consistent with that observed in worldwide studies.

Keywords Japanese · Chronic hepatitis B · Entecavir · Lamivudine refractory · Lamivudine resistant

Introduction

Chronic hepatitis B (CHB) infection is a global public health problem that is estimated to cause between 500,000 and 1.2 million deaths annually [1–3]. Three-quarters of all chronically infected individuals live in the Asia–Pacific region, where hepatitis B virus (HBV) is the leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [4]. In Japan, the prevalence of HBV infection was estimated to be 0.8% in 2000, and the vast majority of individuals are infected with HBV genotype C [4–6]. Genotype C virus has been associated with high rates of progression to the complications of CHB, including cirrhosis and HCC [7–11]. In addition to genotype, the level of HBV DNA in the serum is strongly associated with liver disease progression [12, 13]. Persistently detectable and elevated viral loads predict the highest risk of progression to cirrhosis and HCC [12–14]. Suppression of HBV replication with antiviral therapy may reduce the risk of complications and improve the long-term outcomes of CHB patients [15].

Lamivudine has been widely used for the treatment of CHB since its development and initial approval 10 years ago [16, 17]. Lamivudine has demonstrated efficacy and long-term safety and was shown to result in histologic improvement when administered for up to 3 years [16, 18, 19]. However, resistance to lamivudine emerges at a rate of approximately 20% per year and is found in approximately 70% of patients after 4 years of therapy [20, 21]. The emergence of lamivudine resistance may be associated with increases in HBV DNA and alanine aminotransferase (ALT) levels, and loss of histologic response [16, 18, 22]. In patients with cirrhosis, lamivudine resistance may lead to hepatic decompensation and HCC [15, 23, 24]. Recently published CHB treatment guidelines no longer recommend lamivudine as first-line therapy for treatment-naive patients because of the problems that resistance introduces in the management of individual patients and the negative impact that lamivudine resistance has on the subsequent use of other 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).

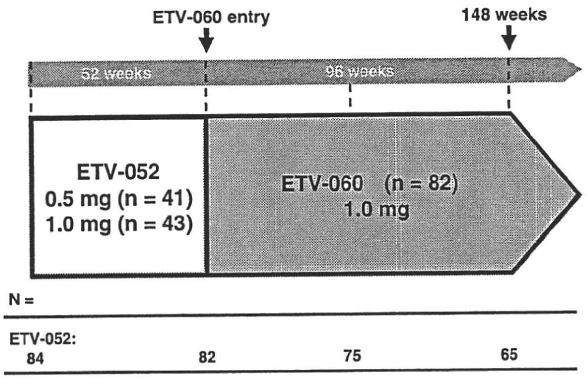

Fig. 1 Lamivudine-refractory, long-term treatment cohort. Eighty-two patients completed 52 weeks of treatment in study ETV-052 and entered rollover study ETV-060, with no interruption or gap in treatment. Sixty-five patients remained on treatment (entecavir 1.0 mg daily) through 96 weeks in study ETV-060,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

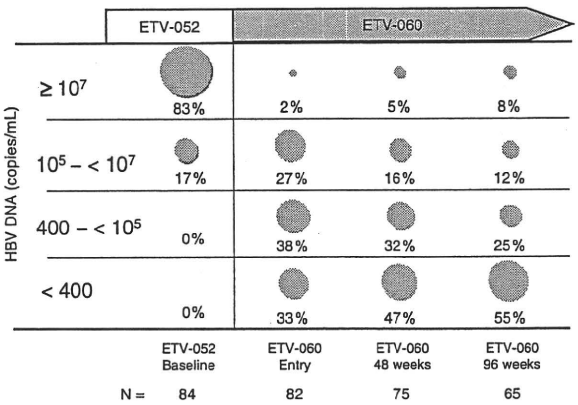

Fig. 2 Distribution of HBV DNA over time in the lamivudine-refractory, long-term treatment cohort. The proportion of patients with HBV DNA of >400 copies/mL increased through ETV-060 week 96 (148 weeks of total entecavir treatment time)

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

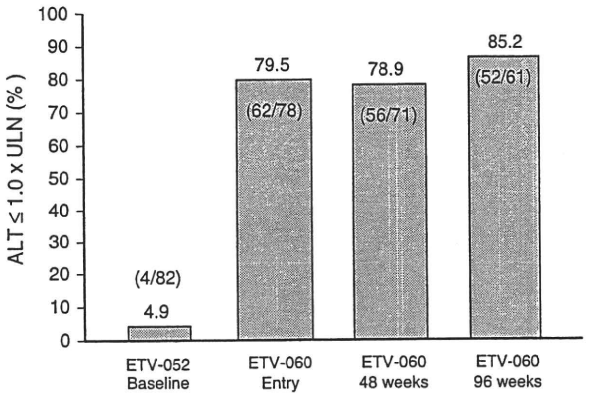

Fig. 3 Proportions of patients with normal ALT (ALT ≤ 1.0 × ULN) over time in the lamivudine-refractory, long-term treatment cohort. Seventy-eight patients had abnormal ALT (ALT > 1.0 × ULN) at pretreatment baseline. At week 96 of study ETV-060, patients had received a total of 148 weeks of entecavir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

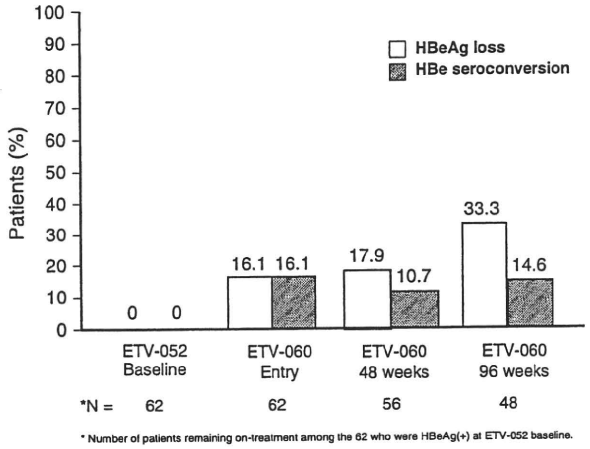

Fig. 4 Proportions of patients with HBeAg loss and HBe seroconversion over time in the lamivudine-refractory, long-term treatment cohort. Sixty-two patients were HBeAg positive at pretreatment baseline. At week 96 of study ETV-060, patients had received a total of 148 weeks of entecavir therapy

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 × 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 ≥1 × 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).

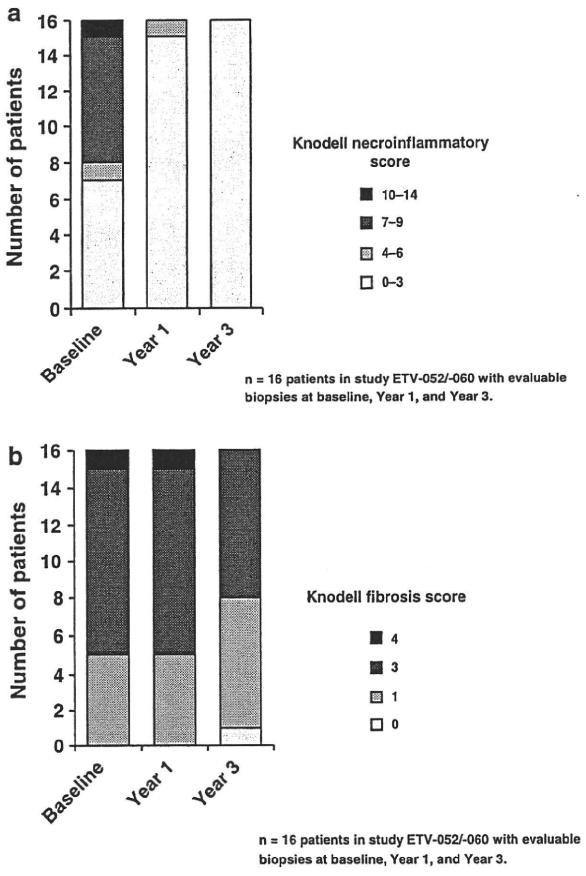

Fig. 5 Distribution of Knodell necroinflammatory scores (**a**) and Knodell fibrosis scores (**b**) at baseline, year 1 (48 weeks), and year 3 (148 weeks) for the 16 patients who had evaluable liver biopsies at all 3 time points

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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